VOLUME 6 MAY 1957 NUMBER 2

AUSTRALASIAN ANNALS OF MEDICINE

Journal of The Royal Australasian College of Physicians

CONTENTS

NOTICE TO CONTRIBUTORS

AUSTRALASIAN ANNALS OF MEDICINE is intended for the publication of original observations and research in internal medicine and the medical sciences in general by medical graduates and other scientific workers.

Articles submitted for publication are understood to be offered to AUSTRALASIAN ANNALS OF MEDICINE only, unless the contrary is stated.

Articles submitted for publication should be typed with double or triple spacing, with a margin of at least one inch and with adequate space at the top and bottom of each sheet. Carbon copies should not be sent.

References to articles and books should be carefully checked. They should conform to the Harvard System, appearing in the text as author's name, followed by the year of publication, and listed alphabetically at the end with the following particulars: surname of author, initials of author, year, full title of article, name of journal abbreviated in the style of the "Quarterly Cumulative Index Medicus", volume, number of first page of the article. If a reference is made to an abstract of a paper, the name of the original journal, together with that of the journal in which the abstract has appeared, should be given with full particulars in each instance.

All measurements should be expressed in the metric system.

Line drawings, charts, graphs and so forth should be drawn on thick white paper or special graph paper in Indian ink. Photographic prints should be reproduced clearly on glossy paper. Skiagrams can be reproduced only if good prints are available. In the journal, figures will as a general rule be either 7·5 or 15 centimetres wide, approximately. Lettering and other details should therefore be sufficiently large to allow such reduction without loss of clarity. The number of illustrations sent should be restricted as far as possible. Only a limited number are accepted without the making of special arrangements.

Reprints can be supplied at cost price; the minimum number is 50 copies. Orders for reprints should be given when the author's proof is returned.

Editorial communications should be addressed to the Honorary Secretary of the Editorial Committee, The Royal Australasian College of Physicians, 145 Macquarie Street, Sydney.

The journal is published quarterly in February, May, August, and November. The subscription rate is four pounds per annum (one pound per issue), payable in advance to The Royal Australasian College of Physicians, 145 Macquarie Street, Sydney. Advertising rates may be obtained on application to Australasian Medical Publishing Company Limited, Seamer and Arundel Streets, Glebe, Sydney.

AUSTRALASIAN ANNALS OF MEDICINE

VOLUME 6 MAY, 1957 NUMBER 2

THE ÆTIOLOGY OF PITUITARY TUMOURS : THE ROLE OF HYPOGONADISM AND HYPOTHYROIDISM[1]

J. E. CAUGHEY[2]

From the Department of Neurology, University of Otago, New Zealand

SUMMARY

In rodents partial or complete impairment of thyroid and also of gonadal function leads in time to the development of pituitary tumours.

There is evidence in the literature of the development of basophile tumours in humans who die of myxœdema.

In no case presenting as a pituitary tumour in our series of 50 cases has previous hypothyroidism appeared to be significant.

In two cases quoted from Cushing's series, and in four personal cases in which pituitary tumours developed, there had been a complete or partial gonadal failure for many years preceding the signs and symptoms of tumours.

It is suggested that in humans, as in rodents, the formation of some pituitary tumours may follow overactivity of gonadotrophic cells secondary to hypogonadism.

SEVERAL workers have reported changes in the pituitary glands of rats and mice, ranging from an increase in basophile cells to the formation of adenomata, following prolonged deficiency of thyroid hormone.

Pituitary tumours occur in certain strains of mice given sufficient I^{131} to destroy the thyroid gland. Gorbman (1949) found that 80 to 300 microcuries of I^{131} led to enlargement of the pituitary gland which was quite apparent one hundred and fifty days after the administration of the radioactive iodine. Large tumours were present one hundred days later, their size being directly related to the size of dose of the I^{131}. Implantation of thyroid tissue and administration of thyroxine prevented pituitary enlargement and tumour formation in mice treated with doses of I^{131} adequate to form pituitary tumours. Gorbman (1952) concluded that the combination of radioactive iodine and thyroid hormone deficiency were essential factors in the development of the pituitary tumours. Goldberg and Chaikoff (1951) showed that mice treated with 600 microcuries of I^{131} showed marked hyperplasia of basophile cells of the anterior lobe of the pituitary gland. These changes were prevented if dried thyroid extract was given.

Mice treated with thiouracil for 400 days had enlarged pituitary glands but no actual tumour formation (Gorbman, 1949). Pituitary tumours developed in mice (Moore, Brackney and Bock, 1953) and in rats (Purves and Griesbach, 1951) treated with thiouracil, also in rats treated with radioiodine (Doniach, 1953). Purves and Griesbach (1951) and Doniach (1953) reported these as basophile adenomata. Bielschowsky (1955) summarizes the literature and states that thyroid hormone deficiency is the one factor common to these experimentally produced pituitary tumours. The pituitary reacts to the lowered level of thyroid hormone with an increase in T.S.H.-secreting basophile cells. In the course of time in the rat and in mice, pituitary tumours develop. These tumours can be prevented by adequate amounts of thyroid hormone.

Castration, with the resultant overactivity of gonadotrophic basophile cells, does not lead to the production of adenomata with the same regularity as does thyroxine deficiency.

[1] Received on November 30, 1956.

[2] Associate Professor in Neurology.

Under some conditions the gonadotrophic cells appear almost inactive up to eighteen months after castration. Basophile tumours, however, were observed by Griesbach and Purves (1956) in seven out of eight rats castrated at nine months and examined twenty-seven months later. They were accompanied by large numbers of active-looking normal luteinizing hormone-secreting basophile cells (L.H. cells).

In rats which were castrated at birth, Houssay *et alii* (1955) have reported a high incidence of basophile adenomata of the pituitary gland in eighteen months to two years. Griesbach and Purves (1956) have observed adenomata of the pituitary fifteen months after castration of rats six weeks old. Twenty-four adenomata were found in 39 animals examined fifteen months or more after castration, the incidence being the same in males and in females. Most of these adenomata were composed of cells resembling the L.H. cells of the castration pituitary. In two, the cells closely resembled the enlarged follicle-stimulating hormone basophile cell of castration.

Turning to pituitary tumours in humans, we find that there is some scattered evidence of tumour formation following prolonged thyroid hormone deficiency. Means (1948) reports three post-mortem examinations on patients with hypothyroidism. In two of these the pituitary gland was destroyed by tumour, and it was presumed that the myxœdema was secondary to the destruction of the pituitary gland—so-called pituitary myxœdema. In the third case, the pituitary gland appeared normal, but microscopic examination of it showed an "unclassifiable focal lesion in the anterior lobe consisting of neutrophilic undifferentiated cells which the pathologist believed represented an area of focal hyperplasia". Zondek (1944) reported a case of a girl of twenty years with congenital myxœdema and a pituitary tumour. X-ray examination revealed a considerable enlargement of the pituitary fossa. Uyematsu (1920) described the autopsy findings on a patient who had died of untreated myxœdema of ten years' duration. The pituitary gland was slightly smaller than normal; the cells of the anterior lobe were numerous and compact, with acidophile cells increased both centrally and peripherally.

Langeron *et alii* (1954) described two patients with myxœdema who died in coma. In one of these the pituitary was enlarged as a result of diffuse hyperplasia and contained a localized basophile adenoma the size of a pea. The other became comatose and was roused by intravenous administration of thyroxine. He died four days later from a cardio-vascular collapse. At autopsy the thyroid was hardly recognizable and was nearly all fibrous tissue. The pituitary was large and the anterior lobe hypoplastic, and there was a basophile adenoma.

Apart from these few cases, no attempt has been made to correlate hypothyroidism or hypogonadism with the development of pituitary tumours in humans. In an attempt to do this, we have made a search of some of Cushing's (1912) classical case records and also have examined the records of 50 other cases that have been studied in the Dunedin Hospital. As would be expected, in many of these the documentation was inadequate to cover our problem. We found no case in which hypothyroidism appeared to be the forerunner of a pituitary adenoma.

The search for evidence of hypogonadism preceding the development of pituitary tumours proved to be more rewarding. Cushing (1912) quotes two cases, and four personal cases appear to give some support to such a suggestion.

Two Cases from Cushing's "Pituitary Body and its Disorders"

Case I.—Cushing makes reference to a case of a married woman of middle age with evidence of increased intracranial pressure due to a pituitary tumour. There was evidence of incomplete pubescence, the breasts being underdeveloped, and there was no axillary and very little pubic air. Cushing stated that bilateral oophorectomy had been performed some time previously, and the ovaries were reported to be both small and cystic.

Case II.—This was Cushing's Case XIV. A single saleswoman, aged twenty-six years, had had a menstrual period at fourteen years of age and thereafter amenorrhœa. At sixteen she had developed headaches, and at twenty-one these had become severe and persistent.

On examination, her adolescent characteristics were fully developed. She had bilateral optic atrophy and a right upper temporal quadrant defect in the visual field. X-ray examination of the skull revealed a greatly enlarged pituitary fossa. A bitemporal decompression was carried out in order to relieve the intracranial pressure. Later a gynæcologist performed double oophorectomy and reported: "the ovaries show a great number of cysts visible to the unaided eye. Some of these cysts contain shreds of *membrana granulosa*. There are no fully developed Graafian follicles; no primordial ova or Pflüger's tubes; stroma in excess."

Four Personal Cases

Two cases are reported in which bilateral oophorectomy was followed several years later by the development of pituitary tumours.

Case III.—Miss L.M., a masseuse, aged forty-seven years, was admitted to the Dunedin Hospital in 1944 on account of severe headaches and diplopia. At the age of twenty-three she had had hysterectomy and

bilateral oophorectomy on account of severe menorrhagia. She had developed bifrontal headaches fifteen months prior to her admission to hospital. Six months previously, she had noticed diplopia, which persisted, and for two months her vision had been failing in both eyes.

On examination, she was a stout woman with scanty body hair and a dry skin. The visual acuity in the right eye was 6/6 and in the left eye 6/12. There was bilateral optic atrophy, and she had bitemporal hemianopia. X-ray examination revealed an enlarged pituitary fossa with erosion within the fossa. At operation, Mr. M. Falconer did an intracapsular removal of the pituitary tumour, which was reported to be a chromophobe adenoma.

CASE IV.—Mrs. D.S.M.C. was a housewife, aged fifty-seven years, with six children. Twenty-three years previously, when aged thirty-four, she had had hysterectomy and bilateral oophorectomy on account of menorrhagia. There had been complete amenorrhœa and loss of libido since that time. She consulted the author on account of an acute vestibular attack. She had had occasional attacks of vertigo without sensory loss over a period of two years. There was no deafness, and the results of Rinné and Weber tests were normal. For six months she had had intermittent bifrontal and temporal headaches.

On examination, she was a small woman with a sallow complexion. The optic discs were normal, and the visual fields were full. The visual acuity was J18 in the right eye and J16 in the left; corrected it was J2 in the right eye and J4 in the left. X-ray examination of the skull revealed an enlarged pituitary fossa consistent with a pituitary tumour (Dr. C. Begg). The glucose tolerance curve was normal.

In the absence of severe pressure effects, surgery was not considered, and a course of deep X-ray therapy was given to the pituitary gland. After this she remained symptom-free for a year. Then the headaches returned, and further attacks of giddiness occurred. When she was examined, the fundi and fields were still natural, but there was now evidence of hypopituitarism. The basal metabolic rate was 30% on two occasions. The serum lipoid content was 1240 milligrammes per 100 millilitres. The 17-ketosteroid excretion amounted to five milligrammes per day, and the result of the water excretion test was 74%.

Treatment with prednisone, five milligrammes daily, sodium thyroxine, 0·1 milligramme daily, and "Primotestone" depot, 250 milligrammes every two months, has led to marked improvement in her state of health. She is free of headache and vertigo.

In two cases in male subjects relative hypogonadism preceded the development of pituitary tumour.

CASE V.—W.J., a single labourer, aged twenty-seven years, was seen by Mr. Anthony James in April, 1951, on account of headaches and failing vision of six months' duration. He was first aware of this defect when he was unable to see people approaching from the right side. He had always been subject to headaches, but for six months they had been more frequent and more severe. In 1942 he noticed some enlargement of his breasts, and he developed a discharge from the left nipple. For six months the fullness of the breasts had been more apparent. There had been failure of potentia and libido.

On examination, he was a dull apathetic young man. He had bitemporal hemianopia. The visual acuity was 6/12 in the right eye and 6/9 in the left. The optic discs were pale. He had scanty facial hair and very scanty fine hair on the limbs. The pubic hair had a female type of distribution. X-ray examination of the skull showed an enlargement of the pituitary fossa. The result of the water excretion test was 42%.

At operation a pituitary tumour was found and partially removed. It was reported to be a chromophobe adenoma.

CASE VI.—H.L., a married waterside worker, aged thirty-nine years, was seen in May, 1956, on account of failing vision in the right eye of five years' duration. For as long as he could remember he had had little or no body hair, and scanty fine hair on his limbs. At eighteen he had had measles and mumps without obvious orchitis. He had commenced shaving at eighteen and at the time of examination shaved every third day. He had married at the age of twenty-five and had one child, now aged twelve. His potentia and libido had never been great and for three years had failed completely.

He had a thin skin, very scanty facial hair and scanty hair on his limbs. The distribution of pubic hair was of a female type. Both optic discs were pale, and he had a quadrant defect in the right temporal field. The result of the water excretion test was 22%. X-ray examination of the skull revealed an enlarged pituitary fossa.

At operation a right frontal craniotomy was performed, and portion of a pituitary adenoma was removed; this was reported to be a chromophobe adenoma.

Substitution therapy with cortisone, 25 milligrammes daily, thyroxine, 0·1 milligramme twice a day, and "Primotestone" depot, 250 milligrammes every six weeks, resulted in a marked improvement in his physical state with a return of energy, a loss of apathy and a return of potentia and libido.

DISCUSSION

It is apparent that, in rats and in mice, ablation of the thyroid gland by antithyroid drugs or by radioactive iodine leads to increased activity of the basophile cells of the pituitary gland and sometimes to pituitary adenomata.

In humans there is the evidence of the formation of basophile adenomata in the two cases reported by Langeron *et alii* (1954). Uyematsu (1920) reported a central and peripheral increase in acidophile cells. In the two cases with pituitary adenomata quoted by Means (1948), it was presumed the pituitary tumour preceded the myxœdema. It is not impossible that in one of these cases the hypothyroid state may have preceded the pituitary tumour. In Zondek's (1944) case the same query must exist, as to whether the myxœdema was a forerunner of the pituitary tumour or *vice versa*.

A search of our own and of some of Cushing's case records has not revealed any cases of pituitary tumour which might have been preceded by a state of hypothyroidism. This is not surprising, as doctors are now well alive to the hypothyroid syndrome, and treatment is

usually instituted early. It is notable that in rats and mice tumours can be prevented by giving thyroid hormone. Thus, in recent years, severe untreated myxœdema of prolonged duration is a rare phenomenon.

In rats and mice it is clearly established that ablation of the gonads by surgery leads in time to the development of pituitary tumours, which modern staining clearly defines as basophile adenomata. In the human cases quoted from Cushing's records (1912) the clinical evidence suggests a degree of hypogonadism, which we assume was due to the cystic state of the ovaries in both cases. It may be argued that this was secondary to a primary pituitary lesion. In two of our cases bilateral oophorectomy had been carried out several years before the development of pituitary tumours. In such cases it is possible that the adrenal cortex may take over the œstrogenic function. In the second case (Case IV) at least, this seemed unlikely, as there had been complete loss of libido following operation. In the two male patients, the sequence does not appear to be so clear. In one (Case V), the development of gynæcomastia and the scanty secondary sex hair suggest failure of spermatogenesis in the presence of androgenic secretion. In Case VI, measles and mumps may have impaired the function of the gonads, although there is evidence that the growth of facial hair was defective before this. It is a clinical fact that in many cases of pituitary tumour of the chromophobe type in males there is often evidence of defective secondary sexual hair growth for many years before the patient presents with symptoms and signs of a pituitary tumour; this suggests a prolonged period of defective androgenic function preceding the development of tumour. Thus it appears that in some such clinical cases a good case can be made for regular substitution therapy with androgens; and in known cases of hypogonadism in males and females, the result of surgery or disease, regular substitution therapy should be commenced.

It will be noticed that in three of our cases the type of tumour was clearly chromophobe, whereas the reports on rats and mice were of basophile tumours. This has to be viewed in the light of modern cytological study of the pituitary. It is recognized that the chromophobe cell is an inactive cell and may be a quiescent phase of an acidophile cell. With modern staining methods, chromophobe cells are often found in patients with clearly defined acromegaly; this suggests that the acidophile cell may be transformed to a chromophobe cell. The same state of affairs could hold for the basophile series. Purves and Griesbach (personal communication) have found that 60% of a group of rats, of the same age and sex and castrated at the same time, had developed pituitary tumours. On examination and section, these were found to be at varying stages of development. In some, the tumour was small and made up of basophile cells only. In others, tumour formation was more advanced and contained chromophobe type cells, but with basophilic or acidophilic elements still apparent in the tumour cells. The basophile tumours in the rodents were found at an early stage of tumour formation. It would be of interest if similar groups of animals were sacrificed after another year to note if the tumours were then of the chromophobe type.

In all six cases reported, it is suggested that there was evidence of complete or partial failure of the gonads; and if we draw on the analogy with the animal experiments, it seems possible that the development of the pituitary tumours was due to an overactivity of gonadotrophic cells, with subsequent tumour formation.

ACKNOWLEDGEMENTS

It is a pleasure to thank Mr. Anthony James for giving me access to his case records, Dr. W. Griesbach and Dr. F. Bielschowsky for their advice and assistance, and Miss J. Smith for secretarial assistance.

BIBLIOGRAPHY

BIELSCHOWSKY, F. (1955), "Neoplasia and Internal Environment", *Brit. J. Cancer*, **9**, 80.

CUSHING, H. (1912), "The Pituitary Body and its Disorders", J. B. Lippincott Company.

DONIACH, I. (1953), "The Effect of Radioactive Iodine Alone and in Combination with Methylthiouracil upon Tumour Production in Rat's Thyroid Gland", *Brit. J. Cancer*, **7**, 181.

GOLDBERG, R. C., and CHAIKOFF, I. L. (1951), "On the Nature of the Hypertrophied Pituitary Gland Induced in the Mouse by I^{131} Injections, and the Mechanism of its Development", *Endocrinology*, **48**, 1.

GORBMAN, A. (1949), "Tumorous Growths in the Pituitary and Trachea Following Radiotoxic Dosages of I^{131}", *Proc. Soc. Exp. Biol. & Med, N.Y.*, **71**, 237.

GORBMAN, A. (1952), "Factors Influencing Development of Hypophyseal Tumors in Mice After Treatment with Radioactive Iodine", *Proc. Soc. Exp. Biol. & Med., N.Y.*, **80**, 538.

GRIESBACH, W. E., and PURVES, H. D. (1956), "Basophil Cell Adenomas in the Rat Pituitary", *Proc. Univ. Otago Med. School*, **34**, 1.

HOUSSAY, B. A., HOUSSAY, A. B., CARDEZA, A. F., and PINTO, R. M. (1955), "Estrogenic Adrenal Gland Tumors and Pituitary Gland Tumors in Castrated Animals", *Schweiz. med. Wchnschr.*, **85**, 291.

LANGERON, L., GIARD, P., and VINCENT, G. (1954), "Myxœdema with Basophil Adenoma of Hypophysis", *J. sc. méd.*, Lille, **72**, 7.

MEANS, J. H. (1948), "The Thyroid and its Diseases", 2nd Edition, J. B. Lippincott Company.

MOORE, G. E., BRACKNEY, E. L., and BOCK, F. G. (1953), "Production of Pituitary Tumors in Mice by Chronic Administration of Thiouracil Derivative", *Proc. Soc. Exp. Biol. & Med., N.Y.*, **82**, 643.

PURVES, H. D., and GRIESBACH, W. E. (1951), "The Site of Thyrotrophin and Gonadotrophin Production in the Rat Pituitary Studied by McManus-Hotchkiss Staining for Glycoprotein", *Endocrinology*, **49**, 244.

PURVES, H. D., and GRIESBACH, W. E. (1956), personal communication.

UYEMATSU, S. (1920), "A Case of Myxedematous Psychosis, Clinical and Pathological Report", *Arch. Neurol. & Psychiat.*, **3**, 252.

ZONDEK, H. (1944), "The Diseases of the Endocrine Glands", Edward Arnold and Company, London.

THE NATURE OF THE PIGMENTARY DISTURBANCE IN ADDISON'S DISEASE[1]

BRYAN HUDSON[2] AND G. A. BENTLEY[3]

From the Baker Medical Research Institute and Alfred Hospital Clinical Research Unit, Melbourne

SUMMARY

It is suggested that the pigmentary disorder in Addison's disease results from an excessive production of pituitary melanocyte-stimulating hormone.

The hormones were assayed by the use of intact toads, where concentration was high (for example, pituitary extracts) and of isolated toad skin for low concentrations (for example, blood). A method for isolation of the hormone from blood is outlined.

With these methods it has been shown that in persons with Addison's disease an excessive amount of melanocyte-stimulating hormone is present in the blood, and that this decreases after treatment with cortisone or its derivatives. It has also been shown that the blood melanocyte-stimulating hormone concentration is lowered in patients with destructive lesions of the pituitary.

The significance of these findings in relation to disorders of melanin pigmentation is discussed.

MORE than one hundred years ago, Thomas Addison (1855) described a " peculiar change " in the skin colour of patients " with a diseased condition of the suprarenal capsules ". Since this original description, abnormal pigmentation of the skin and mucous membranes has proved to be a common and conspicuous physical sign of the disease that now bears his name. Although it is known that this change results from an excessive production of melanin by the epidermal melanocytes, there has been little understanding concerning the factors which regulate this production (Lerner and Fitzpatrick, 1950). The purpose of this paper is to describe a series of experiments which show that the pigmentary disturbance is associated with an increased amount of circulating melanocyte-stimulating hormone (MSH) and to discuss the relationship of this hormone to other pigmentary disturbances. The results of the experiments that are described in this paper are in agreement with those recently reported by Lerner, Shizume and Bunding (Lerner *et alii*, 1954; Shizume and Lerner, 1954).

The role of the pituitary in the control of skin colour in lower vertebrates has been appreciated for more than forty years. Two American biologists, P. E. Smith and B. M. Allen, demonstrated independently that the removal of the pituitary from tadpoles was followed by lightening of skin colour (Smith, 1916; Allen, 1916). Three years later, Atwell (1919) showed that darkening of the skin occurred if tadpoles were immersed in an environment that contained extracts of the *pars intermedia* of the pituitary. Since these initial observations many contributions have been made to the understanding of the control of skin colour in lower vertebrates. A substantial number of these contributions came from Hogben and his pupils over a period of nearly twenty years. Their studies included many aspects of the action of MSH: methods for its assay and extraction from pituitary tissues, and an elegant series of observations on the control of the pigmentary effector system in these animals (Hogben and Winton, 1922; Hogben, 1923*a*, 1923*b*; Hogben and Gordon, 1930; Hogben and Slome, 1931, 1936; Hogben and Landgrebe, 1939; Waring, 1940). Other workers have participated in this field; thus, Zondek and Krohn (1932*a*, 1932*b*) described the isolation of this hormone from the intermediate lobe of the toad pituitary.

[1] Received on October 30, 1956.

[2] Research Assistant, Baker Medical Research Institute and Alfred Hospital Clinical Research Unit; now Assistant Physician, Diabetic and Metabolic Unit, Alfred Hospital, Melbourne. This work has been performed in partial fulfilment of the requirements of the University of Melbourne for the degree of Doctor of Philosophy. It was supported, in part, by a grant from the Australian National Health and Medical Research Council.

[3] Pharmacologist, Baker Medical Research Unit. Present address: Nicholas Institute for Medical and Veterinary Research.

Little attention was paid to the possible relationship between the hormonal control of skin colour in lower vertebrates and disorders of pigmentation in man. As far as we are aware, the earliest suggestion of the possible relationship between the pituitary and certain pigmentary disturbances in man was made by Moehlig and Bates (1933). They contrasted the pigmentation that may be observed in conditions associated with pituitary hyperfunction with the skin pallor of patients with partial or complete pituitary destruction. Sheehan, in 1939, made the pertinent observation that, in spite of the pronounced adrenal atrophy observed in patients with destructive lesions of the pituitary, pigmentation of the Addisonian type was never encountered (Sheehan, 1939).

Interest in this problem was revived by the introduction of corticotrophin (ACTH) into clinical medicine. Sprague *et alii* (1950) described the development of abnormal pigmentation in a man, suffering from rheumatoid arthritis, who had received between eight and nine grammes of ACTH over an eighty-seven-day period. This pigmentation was qualitatively similar to that seen in Addison's disease, and the batch of ACTH which had been used in his treatment was shown, subsequently, to darken the skin of frogs. Independently, our interest in this problem was stimulated by a similar observation in a female patient, suffering from rheumatoid arthritis, who had received approximately four grammes of ACTH over a five-week period. This patient developed brownish pigmentation of the skin in the palmar and digital creases. This pigmentation faded, and ultimately disappeared, within two months of the cessation of treatment by ACTH. The concept that the pigmentary disturbance in Addison's disease resulted from uncontrolled pituitary overactivity in the absence of adrenocortical hormones was further strengthened by the observation that treatment with cortisone or hydrocortisone caused a consistent and conspicuous reduction in pigmentation in all patients (Hall *et alii*, 1953). The synthetic mineralocorticoid, desoxycorticosterone, appears to have no capacity to influence skin pigmentation.

Substantial proof of this hypothesis has recently been published by Lerner *et alii* (1954). By a careful series of experiments they have shown beyond reasonable doubt that the increased pigmentation in Addison's disease is associated with an excessive secretion of MSH by the pituitary. Utilizing the technique of assaying this hormone on isolated frog's skin, they have succeeded in demonstrating that the hormone is present in small amounts in the blood and urine of normal patients, and that it is increased in amount in patients suffering from Addison's disease and decreased or absent in hypopituitarism. The parenteral administration of pure preparations of this hormone was associated with a pigmentary disturbance similar to that seen in Addison's disease. Hudson and Bentley (1955) have demonstrated the presence of this hormone in the human pituitary.

Materials and Methods

Assays

A detailed description of the assay methods used in this series of investigations has been given (Hudson and Bentley, 1956).

For the assay of samples in which the hormone concentration was high, live male *Xenopus lævis* toads were used. The material for assay was injected into the dorsal lymph sacs of light-adapted toads and the degree of melanocyte stimulation estimated by the microscopic observation of the melanocytes in a web of the hind paw ninety minutes after the injection. An arbitrary scale of melanocyte stimulation similar to that originally described by Hogben and Gordon (1930) was used. For each assay at least 16 animals were used, the assay being two-point in design with two dose levels of unknown and standard and four toads at each dose level. The maximum sensitivity of this method of assay was such that it enabled the detection of MSH in a concentration of 13 microgrammes per millilitre of our standard powder (Commonwealth Serum Laboratories corticotrophin, batch P.60). The use of hypophysectomized toads increased the sensitivity of the assay some fivefold.

Neither method of assay was sufficiently sensitive for the detection of the small amounts of MSH present in blood. Therefore, a method of assay involving the use of isolated strips of fresh toad skin was developed. Such strips become darker when immersed in solutions containing MSH, and this darkening can be measured by a suitable photoelectric device. For these studies the isolated skin was mounted on a small "Perspex" chamber (Figure I), into which the solution under test was introduced. The degree of darkening was measured by the change in reflectance from the surface of the skin using an E.E.L. reflectance meter. The apparatus is shown in Figure I, and the details of the assay have been published elsewhere (Hudson and Bentley, 1956).

This method is more tedious and much less precise than that which uses living animals, but a 100- to 150-fold increase in sensitivity is obtained, which was quite sufficient to detect the small amount of MSH present in the blood of the patients studied.

Extraction of Hormone from Biological Material

A comparison of some of the different methods of recovery of hormone from blood has been presented (Bentley and Hudson, 1956). The method that was consistently used in this series of observations involved an initial protein precipitation and hormone extraction by acetone and aqueous acetone. The acetone was then removed under reduced pressure at 45° C. The residual aqueous extract was acidified with acetic acid, so that it was equivalent approximately to 0·1N acetic acid. To this extract were added 200 milligrammes of oxycellulose (12% COOH), and the resultant mixture was rotated on a slowly revolving drum for at least twenty-four hours. The oxycellulose fraction was then collected on a coarse sintered glass funnel and washed with 20 to 50 millilitres of 0·1N acetic acid. Three to four millilitres of 0·1N hydrochloric acid were then added to the oxycellulose, and the hormone was eluted by shaking for an hour or more. The eluate was collected, its pH was adjusted to approximately 6·8 (bromthymol blue papers) and it was made up to 10 millilitres with a modified Ringer's solution described by Landgrebe and Waring (1944). Some isoelectric precipitation was not uncommon with adjustment of pH: any precipitate that formed was removed by centrifugation. When prepared in this manner, one millilitre of this solution was equivalent to five millilitres of blood, and a series of experiments in which a known amount of hormone was added to blood yielded recoveries between 70% and 85% of added hormone (Bentley and Hudson, 1956).

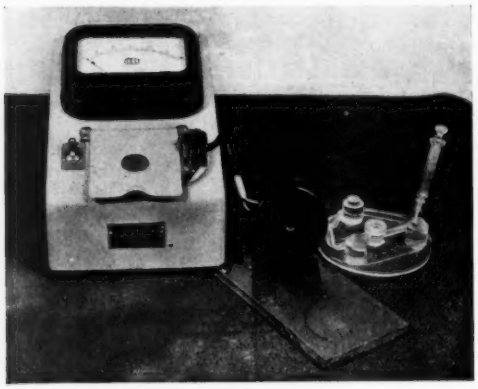


FIGURE I

The apparatus used for the assay of MSH by the isolated toad skin method. On the right is the " Perspex " skin holder, in the centre the reflectance head, and on the left the galvanometer for measuring changes in reflectance.

Clinical Studies

Three groups of persons were studied: nine normal subjects, three hospital patients without evidence of either endocrine or pigmentary disturbance, and four patients with Addison's disease, two with pituitary insufficiency and one pregnant woman. The diagnosis of Addison's disease and pituitary insufficiency was made by conventional methods—history and physical examination and the estimation of urinary corticosteroids and ketosteroids both before and after adrenocortical stimulation with ACTH. In all instances, the response of circulating eosinophil cells to ACTH was determined as a measure of adrenocortical reserve.

TABLE I

The Blood Melanocyte-Stimulating Hormone Concentration of a Series of Twelve Persons Without any Endocrine or Generalized Pigmentary Disorder

Males		Females	
Patient	Concentration (μg/100 ml.)	Patient	Concentration (μg/100 ml.)
Normal	1·5	Normal ..	1·7
Normal	1·3	Normal ..	1·6
Normal	2·4	Normal ..	2·1
Hypertension ..	2·0	Normal ..	1·8
Melanoma ..	1·7	Hypertension ..	2·3
Normal	1·8	Normal ..	1·9
Average ..	1·8	Average ..	1·9

RESULTS

Normal Values

Normal values are shown in Table I and are expressed in microgrammes per 100 millilitres of blood of Commonwealth Serum Laboratories corticotrophin (Batch P.60). It may be noted that the blood level of females is slightly, but not significantly, higher than that of males.

Patients with Endocrine or Pigmentary Disorders

Table II gives brief clinical notes and the concentration of MSH in the blood of patients with endocrine or pigmentary disorders. It

will be noted that in patients with untreated Addison's disease the blood levels are significantly higher than normal, this level falling to the normal range on three occasions in two patients after treatment with cortisone or one of its derivatives. The blood level of the patient, who was five-months pregnant, was also above the normal range. By contrast, two patients with destructive pituitary lesions and marked skin pallor showed subnormal concentrations of MSH in the blood.

TABLE II

The Blood Melanocyte-Stimulating Hormone Concentrations of Two Patients with Addison's Disease Before and After Treatment with Cortisone or One of its Derivatives and Two Others Untreated, of One Pregnant Patient and of Two Patients with Destructive Pituitary Lesions

Patient	Clinical Condition	Blood Levels (μg/100 ml.)
L.F.	Untreated Addison's disease	5·4
L.F.	Addison's disease. Four weeks' therapy with oral cortisone 37·5 milligrammes per day	1·8
L.F.	Addison's disease. In crisis deeply pigmented	6·0
L.F.	Addison's disease. Five weeks' therapy with fluorohydrocortisone acetate. 1·0 milligramme per day	1·8
C.P.	Untreated Addison's disease	7·4
C.P.	Addison's disease. Three months' treatment by oral hydrocortisone 10·0 milligrammes twice a day	1·7
F.O'K.	Untreated Addison's disease	5·3
M.K.	Untreated Addison's disease	7·2
L.O'B.	Five-months pregnant	2·7
L.M.	Post-partum pituitary necrosis	0·2
G.B.	Chromophobe adenoma of the pituitary. Marked pallor of skin	0·4

Units of MSH

At the present time there is no accepted international unit of MSH. Shizume *et alii* (1954) have defined their own scheme of units, one unit being equivalent to 0·04 microgrammes of the powder prepared by the aqueous extraction of beef posterior lobe powder. In this present series our results have been expressed in terms of the weight of Commonwealth Serum Laboratories corticotrophin (Batch P.60). Determination of the potency ratio of this preparation against Lerner's powder gave a value of 1·04. Conversion of our results (microgrammes per 100 millilitres) from normal persons to units (Lerner's units) gives the following values: 192 units per 100 millilitres (males) and 204 units per 100 millilitres (females).

DISCUSSION

Melanin Synthesis

It is generally agreed that the production of melanin results from the oxidation of tyrosine by the copper-enzyme complex tyrosinase. The initial phase of this reaction is the conversion of tyrosine to a diphenol compound with subsequent dehydrogenation of this product to a series of quinone intermediates, the immediate precursor of melanin being indole 5,6-quinone. This reaction occurs in the cytoplasm of the melanocytes, which are located in the skin at the junction of the dermis and epidermis. These, or similar cells, are also present in the eye and the meninges. The formation of melanin, therefore, is a function of the concentration of three substances—namely, the substrate tyrosine, the enzyme tyrosinase and molecular oxygen.

Factors Influencing Melanin Synthesis

Local Factors.—There is some correlation between clinical abnormalities of skin pigmentation and changes in the melanocytes responsible for melanin formation. Thus, in albinism there is a congenital absence of tyrosinase from the cytoplasm of these cells. In certain other conditions there is inactivation of copper ions from the tyrosinase complex with subsequent depression of melanin formation. Lerner has shown, for instance, that sulphydryl groups can combine with the copper of the tyrosinase complex and inhibit its activity. Using histochemical techniques, Goldblum *et alii* (1954) have shown a relative deficiency of sulphydryl groups in the region of the hair bulbs of white skin. This perhaps affords an explanation as to the ability of black hair to grow from white skin. Destruction of sulphydryl groups in inflammatory processes or their oxidation by ultraviolet light is probably the basis of the increased melanin formation that follows inflammation or exposure to ultraviolet light (sun tanning).

Hormönal Factors.—There is abundant evidence, both in lower animals and in man, to associate hormones with the regulation of skin pigmentation. The original observations of Allen and Smith on the influence of hypophysectomy on the skin colour of tadpoles has already been cited. There are other examples of endocrine disturbances and aberrations in pigmentation. Thus in pregnancy some abnormality in skin pigmentation is frequently encountered. Lerner (1955) has shown that there is a progressive rise in the MSH content in pregnant patients with a sharp drop in the early post-partum period. The one pregnant patient in the series was found to have a slight excess of circulating MSH (2·7 microgrammes per 100 millilitres). In thyrotoxicosis increased pigmentation is sometimes seen. This is presumed to be due to pituitary overactivity.

The results of the studies of this series of patients leave little doubt as to the influence of pituitary MSH on skin pigmentation. Patients with Addison's disease who were deeply pigmented showed an increase in the concentration of this hormone in the blood. Treatment with cortisone, which has repeatedly been shown to inhibit pituitary activity, is associated with decrease in pigmentation and a corresponding fall in the blood levels of this hormone. Conversely, patients with reduced melanin pigmentation and consequent skin pallor associated with complete or near complete pituitary failure have been shown to have subnormal amounts of circulating MSH. These findings are in complete accord with those recently reported by Lerner *et alii* (1954).

MSH is not the only hormone influencing skin pigmentation. Treatment of castrate or hypovarian females with œstrogen is commonly associated with increased pigmentation of the nipples. Likewise, the local application of sex hormones to the skins of guinea-pigs and birds may cause increased pigmentation, while the systemic administration of thyroid or œstrogens to birds is frequently associated with colour changes in the feathers. It can readily be shown that adrenaline and noradrenaline inhibit the darkening of toad skin by MSH. At the present time it is not known precisely how any of these hormones influence pigmentation ; whether, for instance, the steroid sex hormones act directly on the melanocytes or exert their effects, as do cortisone and its derivatives, by a central action. Nor is it known what part is played by adrenaline and noradrenaline in human pigmentation.

In lower animals the effects of MSH are well understood. Injection of this hormone into an intact animal is accompanied by a redistribution of the pigment in the pigment-containing cells. In the human it is presumed that this hormone accelerates melanin synthesis within the melanocytes, but in higher vertebrates, under normal circumstances, this hormone would appear to play a permissive rather than a dominant role in affecting skin colour.

During the past five years there has been considerable controversy as to the identity of this hormone. Since it was observed that treatment with ACTH was associated with darkening of the skin in humans, and that injection of minute amounts of ACTH preparations would cause darkening of the skin of frogs, it was claimed that MSH and ACTH were identical. These claims, made by Sulman (1952*a*) and by Johnsson and Högberg (1952), were based upon the observations that adrenal ascorbic acid-depleting and melanophore-expanding properties were destroyed by similar substances, such as trypsin, formalin and certain tissue homogenates, and that they possessed similar chromatographic behaviour when a somewhat non-specific phenol-water system was used. It was also claimed that MSH possessed corticotrophic properties with respect to eosinophil depression, electrolyte excretion and steroid hormone output. Sulman (1952*b*) claimed that the measurement of colour changes in frogs formed a simple method for the assay of corticotrophin. These claims were refuted by Morris (1952) and by Li and his colleagues (Geschwind *et alii*, 1952 ; Reinhardt *et alii*, 1952). They found no positive correlation between adrenal ascorbic acid-depleting, adrenal weight-maintaining and melanocyte-stimulating properties. These findings were based upon differences in reaction to alkali treatment, separation of properties by discontinuous pH gradient on oxycellulose columns, paper electrophoresis and preparation of MSH free of adrenal ascorbic acid-depleting properties by countercurrent distribution with sec-butanol and various aqueous acid solutions.

Simple anatomical separation of the lobes of the pituitary provides additional evidence as to the non-identity of these two hormones. Thus Reinhardt *et alii* (1952) have shown that dissection and extraction of the anterior and intermediate lobes of the pituitary of *Rana catesbiana* yields powders in which the intermediate lobe contains approximately sixty times as much MSH as the anterior lobe, which in turn was more than twice as potent with respect to the adrenal ascorbic acid-depleting factor. Separation of mammalian pituitary into anterior and posterior lobes gives similar dissociation between adrenal ascorbic acid-depleting and melanocyte - stimulating properties. We have found (unpublished observations) that the powder prepared from the posterior lobe by the charcoal adsorption method of Landgrebe *et alii* (1943) is extremely potent with respect to melanocyte-stimulating properties but contains little, if any, adrenal ascorbic acid-depleting properties. Finally, the administration of pure preparations of MSH to humans is associated with no indices of adreno-cortical stimulation when measured by conventional methods—steroid excretion and eosinophil depression (Lerner *et alii*, 1954 ; Salassa *et alii*, 1954).

In the past two years the chemistry of these hormones has been more intensively studied. Lerner and Lee claim to have isolated from

hog pituitary two distinct melanophore hormones, α and β. These are polypeptides with molecular weights of 4000 and contain approximately fifteen different amino acids. These substances are free from conventional corticotrophic effects, and as little as 10^{-10} gramme of α MSH will darken isolated frog skin (Lerner, 1955). Conversely, purified corticotrophin always possesses small, but distinct, melanocyte stimulating properties.

The series of experiments reported in this paper show clearly that in patients with untreated Addison's disease there is an excess of melanocyte-stimulating activity in the blood. We have also shown that this decreases when these patients are treated with cortisone, when there is a fading of the abnormal skin pigmentation. These results are consistent, both qualitatively and quantitatively, with those described by Lerner, who also observed similar changes in urinary excretion. On somewhat insecure grounds it has been assumed that this excessive secretion results from the posterior lobe of the pituitary. If this be true, this is a rather unexpected reciprocal relationship between the adrenal cortex and the posterior lobe, and one which, to our knowledge, is without parallel with respect to other endocrine organs. It is quite possible that these results represent the small but definite melanocyte-stimulating property of corticotrophin, since it has been shown repeatedly that the secretion of this hormone is substantially increased in adrenalectomized animals and patients suffering from Addison's disease.

Acknowledgements

We are indebted to the advice and encouragement given by Professor V. M. Trikojus and Dr. T. E. Lowe throughout the whole of these studies. We also acknowledge the skilled technical assistance of Miss Diane van Assche. Dr. A. B. Lerner kindly provided a supply of pure melanocyte-stimulating hormone, thus enabling us to compare our results with those of his group.

References

Addison, Thos. (1855), "On the Constitutional and Local Effects of Disease of the Suprarenal Capsules", D. Highley, London.

Allen, B. M. (1916), "The Results of Extirpation of the Anterior Lobe of the Hypophysis and of the Thyroid of Rana Pipiens Larvæ", *Science*, **44**, 280.

Atwell, W. J. (1919), "On the Nature of the Pigmentation Changes in the Frog Larva", *Science*, **49**, 48.

Bentley, G. A., and Hudson, B. (1956), "A Comparison of Methods for the Isolation of Melanocyte Stimulating Hormone from Blood", *Australia J. Exper. Biol. & M. Sc.*, in the press.

Goldblum, R. W., Piper, W. N., and Campbell, A. W. (1954), "A Comparison of Three Histochemical Stains for the Demonstration of Protein Bound Sulphydryl Groups in Normal Human Skin", *J. Invest. Dermat.*, **23**, 375.

Geschwind, I. I., Reinhardt, W. O., and Li, C. H. (1952), "Observations on the Connexions between Intermedin and Adrenocorticotropic Hormone", *Nature*, **169**, 1061.

Hall, T. C., McCracken, B. H., and Thorn, G. W. (1953), "Skin Pigmentation in Relation to Adrenal Cortical Function", *J. Clin. Endocrinol. & Metab.*, **13**, 243.

Hogben, L. T. (1923*a*), "A Method of Hypophysectomy in Adult Frogs and Toads", *Quart. J. Exp. Physiol.*, **13**, 177.

Hogben, L. T. (1923*b*), "Studies on Internal Secretion: I. The Effect of Pituitary (Anterior Lobe) Injection upon Normal and Thyroidectomised Axolotls", *Proc. Roy. Soc. London*, Series B, **94**, 204.

Hogben, L., and Gordon, C. (1930), "Studies on the Pituitary: VII. The Separate Identity of the Pressor and Melanophore Principles", *J. Exp. Biol.*, **7**, 286.

Hogben, L., and Landgrebe, F. W. (1939), "The Pituitary Effector System: IX. The Receptor Fields of the Teleostean Visual Response", *Proc. Roy. Soc. London*, Series B, **128**, 317.

Hogben, L., and Slome, D. (1931), "The Pigmentary Effector System: VI. The Dual Characters of Endocrine Co-ordination in Amphibian Colour Changes", *Proc. Roy. Soc. London*, Series B, **108**, 10.

Hogben, L., and Slome, D. (1936), "The Pigmentary Effector System: VIII. The Dual Receptor Mechanism of the Amphibian Background Response", *Proc. Roy. Soc. London*, Series B, **120**, 158.

Hogben, L., and Winton, F. R. (1922), "Studies on the Pituitary: I. The Melanophore Stimulant in Posterior Lobe Extracts", *Biochem. J.*, **16**, 619.

Hudson, B., and Bentley, G. A. (1955*a*), "Melanophore Expanding Activity in Human Pituitary Gland", *Lancet*, **1**, 386.

Hudson, B., and Bentley, G. A. (1956), "The Biological Assay of Melanocyte Stimulating Hormone", *Australian J. Exper. Biol. & M. Sc.*, in the press.

Johnsson, S., and Högberg, B. (1952), "Observations on the Connexion between Intermedin and Adrenocorticotropic Hormone", *Nature*, **169**, 286.

Landgrebe, F. W., Reid, E., and Waring, H. (1943), "Further Observations on the Intermediate Lobe Pituitary Hormone", *Quart. J. Exp. Physiol.*, **32**, 121.

Landgrebe, F. W., and Waring, H. (1944), "Biological Assay and Standardisation of Melanophore Expanding Pituitary Hormone", *Quart. J. Exp. Physiol.*, **33**, 1.

Lerner, A. B. (1955), "Melanin Pigmentation", *Am. J. Med.*, **19**, 902.

Lerner, A. B., and Fitzpatrick, T. B. (1950), "Biochemistry of Melanin Formation", *Phys. Rev.*, **30**, 91.

Lerner, A. B., Shizume, K., and Bunding, I. (1954), "The Mechanism of Endocrine Control of Melanin Pigmentation", *J. Clin. Endocrinol. & Metab.*, **14**, 1463.

Moehlig, R. C., and Bates, G. S. (1933), "Influence of the Pituitary Gland on Erythrocyte Formation", *Arch. Int. Med.*, **51**, 207.

MORRIS, C. J. O. R. (1952), " ACTH and the Pigment Hormone ", *Lancet*, **1**, 1120.

REINHARDT, W. O., GESCHWIND, I. I., PORATH, J. O., and LI, C. H. (1952), " The Significance of Intermedin Activity in Adrenocorticotrophic Hormone Preparations ", *Proc. Soc. Exp. Biol. & Med.*, **80**, 439.

SALASSA, R. M., ALBERT, A., MASON, H. L., POWER, M. H., and SPRAGUE, R. G. (1954), " Comparative Effects of Intermedin and Corticotrophin in Pituitary Insufficiency ", *Proc. Staff Meet. Mayo Clin.*, **29**, 619.

SHEEHAN, H. L. (1939), " Simmond's Disease due to Post-Partum Necrosis of the Anterior Pituitary ", *Quart. J. Med.*, **8**, 277.

SHIZUME, K., and LERNER, A. B. (1954), " Determination of Melanocyte Stimulating Hormone in Urine and Blood ", *J. Clin. Endocrinol. & Metab.*, **14**, 1491.

SHIZUME, K., LERNER, A. B., and FITZPATRICK, T. B. (1954), " In Vitro Bioassay for the Melanocyte Stimulating Hormone ", *Endocrinol.*, **54**, 553.

SMITH, P. E. (1916), " Experimental Ablation of the Hypophysis in the Frog Embryo ", *Science*, **44**, 280.

SPRAGUE, R. G., POWER, M. H., MASON, H. L., ALBERT, A., MATHIESON, D. R., HENCH, P. S., KENDALL, E. C., SLOCUMB, C. H., and POLLEY, H. G. (1950), " Observations on the Physiologic Effects of Cortisone and ACTH in Man ", *Arch. Int. Med.*, **85**, 199.

SULMAN, F. G. (1952*a*), " Chromatophorotrophic Effect of Adrenocorticotrophic Hormone ", *Nature*, **169**, 588.

SULMAN, F. G. (1952*b*), " Simple Test for Blood-ACTH ", *Lancet*, **1**, 1161.

WARING, H. (1940), " The Chromatic Behaviour of the Eel ", *Proc. Roy. Soc. London*, Series B, **128**, 343.

ZONDEK, B., and KROHN, H. (1932*a*), " *Hormon des Zwischenlappens der Hypophyse (Intermedin)* ", *Klin. Wchnschr.*, **11**, 405.

ZONDEK, B., and KROHN, H. (1932*b*), " *Hormon des Zwischenlappens der Hypophyse (Intermedin)* ", *Klin. Wchnschr.*, **11**, 849.

HYPOGLYCÆMIC SULPHONAMIDES IN THE MANAGEMENT OF DIABETES MELLITUS[1]

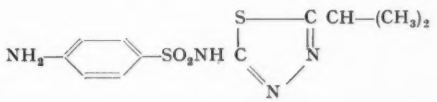
EWEN DOWNIE,[2] JOSEPH BORNSTEIN[3] AND BRYAN HUDSON[3]
From the Diabetic and Metabolic Unit, Alfred Hospital, Melbourne

SUMMARY

A brief review is given of recent claims that certain sulphonylurea compounds possess hypoglycæmic action. The results of clinical trials in the Diabetic and Metabolic Unit of the Alfred Hospital are presented. Clinical trials were undertaken on a selected group of 26 patients (18 females and eight males) and a satisfactory therapeutic response was obtained in 19 patients (13 females and six males). In the remaining patients treatment was regarded as a failure.

The possible mode of action of sulphonylurea drugs is considered, and evidence is presented which suggests interference with transaminase systems in the liver. The conclusion is reached that these drugs do not act as substitutes for insulin, and that in view of side effects and the uncertain sequelæ which may follow their prolonged administration, further work is necessary before they are made available for general use.

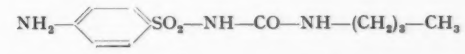
CONSIDERABLE interest has been aroused recently by reports that some sulphonylurea derivatives possess the property of lowering the level of blood sugar in normal human beings. This observation was made originally by Janbon (1942) during treatment of patients suffering from typhoid fever with *p*-aminobenzenesulphonamidoisopropylthiadiazole (I.P.T.D.).



His findings were confirmed by Loubatières (1944), who, as a result of animal experiments, established the fact that the drug was effective only in the presence of pancreatic tissue, and that no hypoglycæmic action could be demonstrated in totally pancreatectomized animals. Loubatières (1955) concluded that I.P.T.D. might have a dual action on the islet tissue of the pancreas, by stimulating secretion of insulin by the β cells and by depressing the secretion of glucagon by the α cells.

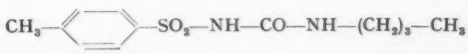
Further work on the relation between the chemical structure of compounds and their blood sugar lowering action suggested that this effect was closely linked with the *p*-aminobenzenesulphonamidothiadiazole structure. The *p*-amino group on the benzene ring appeared to be indispensable, but the aliphatic side chain on the thiadiazole part of the molecule could be modified to some extent without loss of hypoglycæmic action.

No clinical application of this property of these compounds was made for some years, possibly because of the fact that Janbon reported some fatalities in his original series of patients. However, Franke and Fuchs (1955), while testing a new sulphonamide clinically, observed that it also lowered the level of blood sugar in human patients. This substance was N_1-sulphanilyl-N_2-n-butylcarbamide (BZ-55, "Carbutamide", "Nadisan", U 6987).



Franke and Fuchs reported satisfactory control of a number of diabetic patients, some of whom had been able to discontinue the use of insulin. Their results were confirmed by Bertram *et alii* (1955) in a separate clinical trial.

Similar claims were made soon afterwards for another compound, N_1-p-tolylsulphonyl-N_2-n-butylcarbamide (D 860, "Rastinon", "Orinase", U 2043).



[1] Presented at an ordinary meeting of The Royal Australasian College of Physicians, in Melbourne, in October, 1956.

[2] Honorary Physician.

[3] Assistant Physician.

This preparation is, strictly, not a sulphonamide and has practically no bacteriostatic action. However, in all other respects it appears to act like sulphanilyl-n-butylurea. A recent report of extensive clinical trials in a number of German centres presents evidence similar to that of Franke and his colleagues. It has been claimed that there is a correlation between the age of onset of diabetes and the age of a patient and the possibility of response to the drugs. It has been pointed out that diabetics who are young or tall and thin are less likely to respond than are obese patients of middle age. The drugs are contraindicated in all complications of the diabetic state such as ketosis, infections and surgical procedures. Side effects from the prolonged use of the drugs have received small notice in the German literature up to the present time. Bertram reported skin rashes in six out of 82 patients treated with BZ-55, and a slightly lower proportion of skin lesions was noted in association with treatment by D 860. So far little evidence has been presented by German clinicians on depression of white blood cell formation.

Within the past few months, as supplies have become available to workers in other centres, reports of clinical trials in other countries have been published, which allow a preliminary appreciation of the place of these drugs in the management of *diabetes mellitus*. The majority of reports are concerned with the use of BZ-55. There is less evidence available on D 860, although there is no reason to assume that the action or effects of the latter preparation are essentially different.

In considering the results published in the *British Medical Journal* (1956), *The Journal of the American Medical Association* (1956) and *The Canadian Medical Association Journal* (1956) together with our own experiences (1956), it is necessary to appreciate that the principles of treatment of the middle-aged obese patient are probably different in European countries from those which are adopted elsewhere. It seems that in some instances, at least, German patients who are claimed to be satisfactorily controlled by a sulphonamide could be equally well controlled by dietary means if their weights were reduced to normal proportions. It is now established that these drugs lower the blood sugar content of many middle-aged diabetics, and that some who have previously required insulin have been able to substitute oral therapy and remain under control. However, there is no certain knowledge as to how these drugs achieve their effect or as to what their effect is. There is little evidence as to the remote consequences of their prolonged use in the diabetic patient. There is, however, an increasing experience of the incidence of side effects, which, so far as BZ-55 is concerned, is reminiscent of Coburn's experience with the continued use of sulphadiazine over long periods of time. The substitution of a methyl group for the amino group on the benzene ring deprives D 860 of all antibacterial action and might make this compound less likely to depress bone marrow activity. Time alone will determine if this speculation is correct. Meanwhile, a summary of experiences in the United States based on approximately 7000 cases in which the patients were treated with BZ-55 has shown an incidence of toxic side effects in over 5%. Kirtley (1956) has reported several fatalities which are probably related to the continuous use of this drug. It is obvious that further research and experience are needed before it will be possible to determine whether these compounds have a place in the treatment of the human diabetic patient. In the meantime they should be regarded as experimental substances to be used only under close supervision.

BZ-55 has been available to us since January, 1956, and the clinical trials undertaken involved the following observations, all of which were made in the first instance with the patients under strict supervision in hospital. The twenty-four-hour output of urinary glucose and serial estimations of blood sugar were performed by a modification of Somogyi Nelson methods. In some instances determinations of the level of drug in the blood were made to ensure that an effective concentration had been attained. In some, the presence or absence of insulin in the blood plasma was determined using the technique of the isolated rat diaphragm. Leucocyte counts were performed regularly to observe whether any undue depression of bone marrow activity occurred. The dosage used was usually a loading dose of two to four grammes, followed by 0·5 to 1·0 gramme every six to eight hours thereafter.

Clinical trials were undertaken on a selected group of 26 patients, as it was felt, in view of earlier experiences overseas, that no useful purpose would be served by trials on juvenile patients. The following is a summary of our experiences :

Total patients : 26 (females, 18 ; males, 8).
Successes : 19 (females, 13 ; males, 6).
Failures : 7 (females, 5[1] ; males, 2).

[1] In one case glycosuria was controlled, but the drug was stopped on account of a skin rash.

Five of the patients responding successfully to BZ-55 had previously required insulin for control.

In this series granulocytopenia was observed on four occasions, but in no instance was it extreme or persistent; it occurred early and subsided without cessation of treatment. No disturbance of hepatic function was observed, and in those cases in which the conventional tests of liver function were performed the results were within the accepted limits of normality. Five patients developed skin rashes of the type commonly seen in the exhibition of other sulphonamide drugs. In one instance this was of such severity as to warrant cessation of treatment. In nine cases retinitis has either appeared or progressed during the period of observation. In two the retinopathy preceded the trial, and both patients took the drug for only a short time, as they failed to respond. Of the remainder four patients had a pre-existing retinopathy. In one of this group it has progressed rapidly since the taking of BZ-55, and three others have almost certainly developed retinopathy since commencing the trial.

Table I gives details of the series of patients under consideration.

The following case histories are described briefly as they exemplify certain interesting features:

CASE 7.—A man, aged sixty-five years, had a history of diabetes for two years prior to trial. Satisfactory control had been maintained with diet alone until one month before the trial period. For no obvious reason he became tired, lost weight and showed continuous glucosuria. He responded satisfactorily to routine doses of BZ-55. A mild skin rash developed within the first two weeks of treatment and subsided without cessation of the drug. For the past five months he has remained satisfactorily controlled without BZ-55 despite the fact that his dietary carbohydrate has been increased to 250 grammes daily (Figure I).

CASE 9.—A woman, aged fifty-nine years, with a history of diabetes for four years, had been satisfactorily controlled with diet and daily insulin dosage of 28 to 40 units. Shortly before the period of observation she became depressed and received psychiatric treatment with benefit. Before trial with BZ-55, administration of insulin was discontinued, and she excreted 60 to 80 grammes of glucose daily. She showed little response to 2·0 grammes of BZ-55, but responded to 4·0 grammes daily. This was soon reduced to 1·5 grammes and later discontinued. She remained aglycosuric for some weeks, but glycosuria returned on raising her carbohydrate intake to 275 grammes daily. This has been controlled subsequently by 3·0 grammes daily (Figure II).

TABLE I

Carbutamide Trials

Patient		Sex	Age (Years)	Prior Treatment	Result	Complications
1	E.B. (i)	Female	60	Diet two months	Success	
2	E.B. (ii)	Female	64	Diet six weeks	Success	Retinopathy (progressing)
3	T.B.	Male	59	Diet and insulin 16 years	Failure	Acromegaly; control poor with insulin, insulin and BZ-55 and BZ-55 alone
4	V.C.	Female	55	Diet one month	Success	
5	A.E.	Male	64	Diet seven years	Success	Pre-existing peripheral neuritis
6	E.H.	Female	73	Diet six years	Success	Retinopathy
7	R.H.	Male	65	Diet two years	Success (six weeks BZ-55; off BZ-55 four months; still controlled)	Transient skin rash
8	F.K.	Female	58	Diet three months	Success	
9	E.L.	Female	59	Diet and insulin four years	Success	Retinopathy
10	A.R.	Male	32	Diet one year	Success	Mild diabetic retinitis (present before treatment)
11	H.R.	Female	51	Diet six years	Success	Transient skin rash; diabetic retinopathy
12	E.R.	Female	67	Nil	Success	
13	E.T.	Female	56	Diet two months	Success	Mild diabetic retinopathy
14	I.W.	Female	63	Diet six weeks	Success	Transient skin rash
15	E.W.	Female	52	Diet and insulin	Success	Nil (insulin sensitive)
16	A.B.	Male	40	Nil	Success (no BZ-55 for seven months; still controlled)	
17	B.C.	Female	69	Diet nine months	Failure (required N.P.H. 20)	Transient skin rash
18	A.G.	Male	45	Diet and insulin twelve months	Insulin resistance lost during BZ-55 trials; thought to have no effect.	
19	M.W.	Female	64	Diet 12 months	Failure (required insulin)	Pre-existing diabetic retinitis
20	K.W.	Female	19	Nil	Failure (required N.P.H. 60)	Pre-existing asthma
21	I.T.	Female	59	Nil	Glycosuria controlled; BZ-55 stopped because of skin rash	
22	W.M.	Male	63	Insulin six years	Success	
23	E.B.	Female	76	Nil	Success	Pre-existing retinitis
24	F.D.	Male	69	Nil	Success	Pre-existing dementia depression
25	A.N.	Female	76	Nil	Failure (N.P.H. 40)	Pre-existing retinopathy
26	M.B.	Female	64	Diet and insulin (40 units) three weeks)	Success	Nil so far

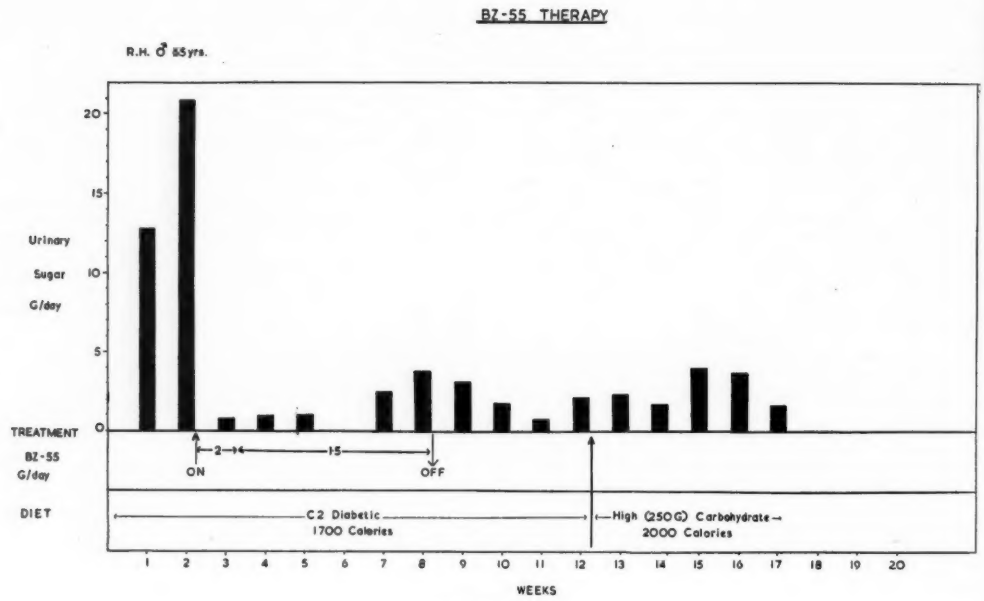


FIGURE I
BZ–55 therapy. Case 7

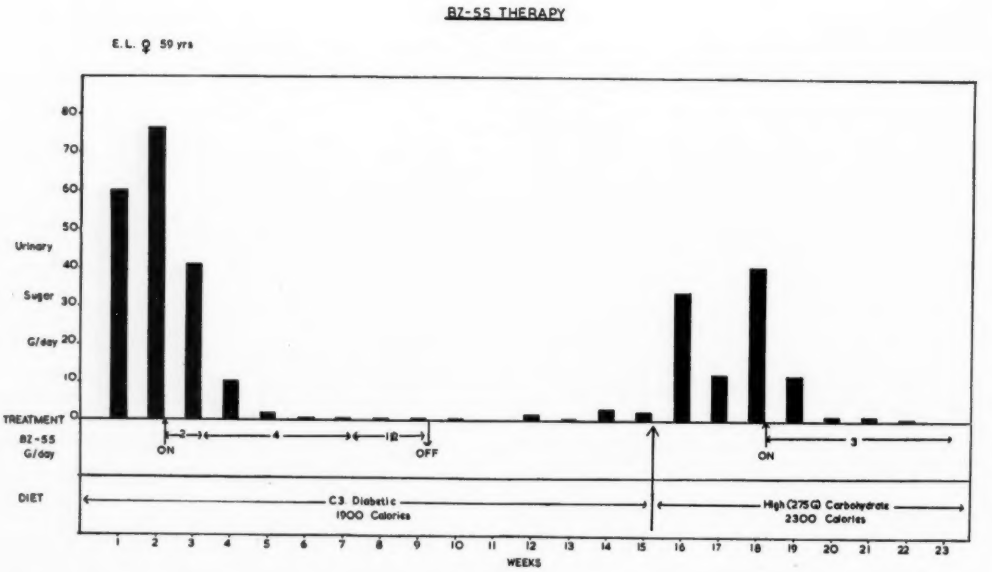


FIGURE II
BZ–55 therapy. Case 9

CASE 10.—A man, aged thirty-two years, with a history of diabetes for twelve years, had persistently refused treatment with insulin and had been uncontrolled by diet during the whole of his diabetic life. He came under our observation because of failing vision, and examination revealed typical diabetic retinitis. His glycosuria was completely controlled by 4·0 grammes of BZ-55 daily, and he has been maintained with 3·0 grammes per day, but reduction to 1·5 grammes daily has failed to exercise control (Figure III).

Adequate amounts of insulin were present in the plasma. She completely failed to respond to BZ-55, but was subsequently satisfactorily controlled with 56 units of isophane insulin daily (Figure V).

CASE 21.—A woman, aged fifty-nine years, gave a history of diabetes for three years prior to trial. A satisfactory response was obtained to a maintenance dose of 4·0 grammes daily, but treatment was discontinued because of a severe skin rash. This was followed by the return of heavy glycosuria, and she has since been controlled satisfactorily with insulin.

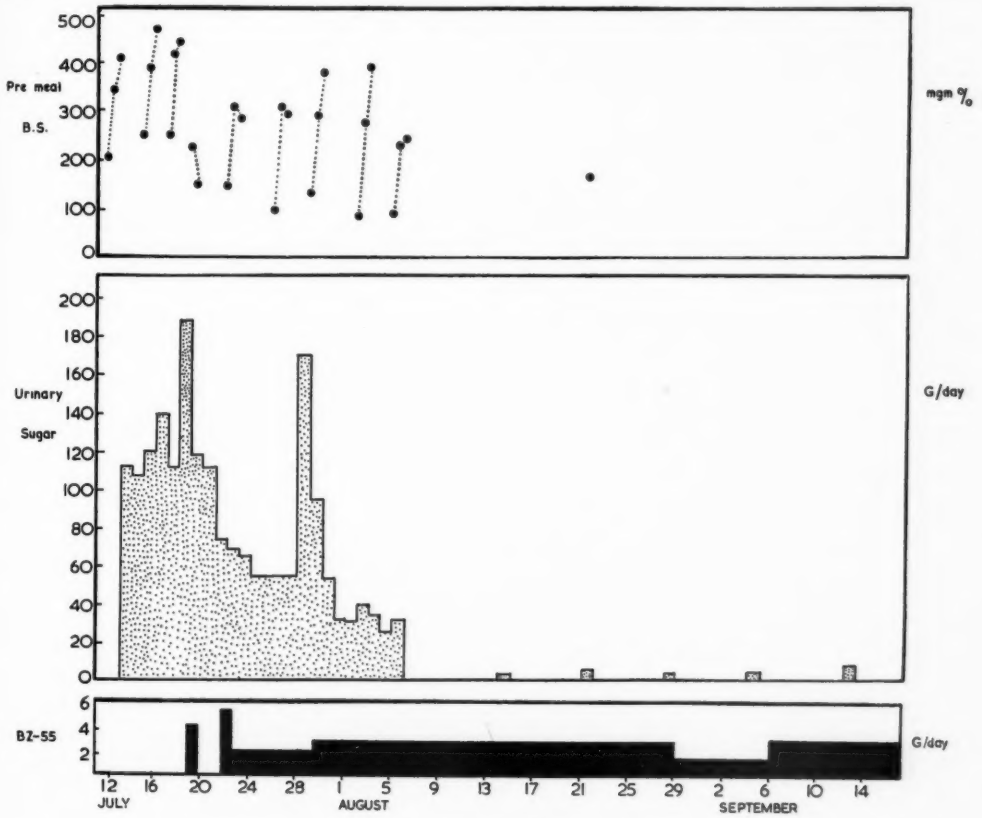


FIGURE III
BZ–55 therapy. Case 10

CASE 17.—A woman, aged sixty-nine years, with a history of diabetes for two years before trial, had been uncontrolled by dietary means. She failed to show satisfactory response to a maintenance dose of 3·0 grammes of BZ-55 per day and has been subsequently stabilized on 20 units of N.P.H. 50 insulin. During treatment she developed a photosensitive rash, which responded to antihistamine ointments (Figure IV).

CASE 20.—A woman, aged nineteen years, with a history of diabetes for one year, had been untreated prior to observation. When admitted to hospital for trial she had marked glycosuria and ketonuria.

If nothing else develops from the observations so far made upon the effect of these sulphonamide drugs on human patients, some fascinating problems have been presented.

The original theories of Loubatières relating to the effect of I.P.T.D. on the islet cells have received much attention. Ferner (1956) propounded the theory that the probable action of these drugs was destruction of α cells with consequent inhibition of the formation of glucagon. Recent studies of human pancreas

(Ferner, 1956) from patients under treatment with BZ-55 have failed to show any evidence to support this contention. The suggestion that stimulation of β cells might result from exhibition of the drug has been studied by Ashworth and Haist (1956) and others. Although there is some suggestion from animal experiments that this may occur in the growing animal, there is no evidence to suggest that this occurs in the human. Mirsky (1956) has postulated that the sulphonylureas act as non-competitive inhibitors of insulinase. Using very high concentrations of BZ-55 he has demonstrated inhibition of the breakdown of insulin *in vitro*. So far it is not certain whether this effect is peculiar to this compound or whether it is a property possessed by all members of the sulphonamide family. Another suggestion is that these substances in some way facilitate the action of insulin, and as a corollary are likely to act only on patients who possess some insulin of their own. Our experiences have failed to support this. BZ-55 has been found to be ineffective in patients with demonstrable insulin in the plasma, and *per contra* it has been effective in one patient in whom no insulin could be demonstrated in the bloodstream. BZ-55 also failed to influence insulin resistance in a patient who had ample insulin demonstrable in his plasma. Young (1956) has suggested that its action may be related to the antibacterial properties of BZ-55, but it is

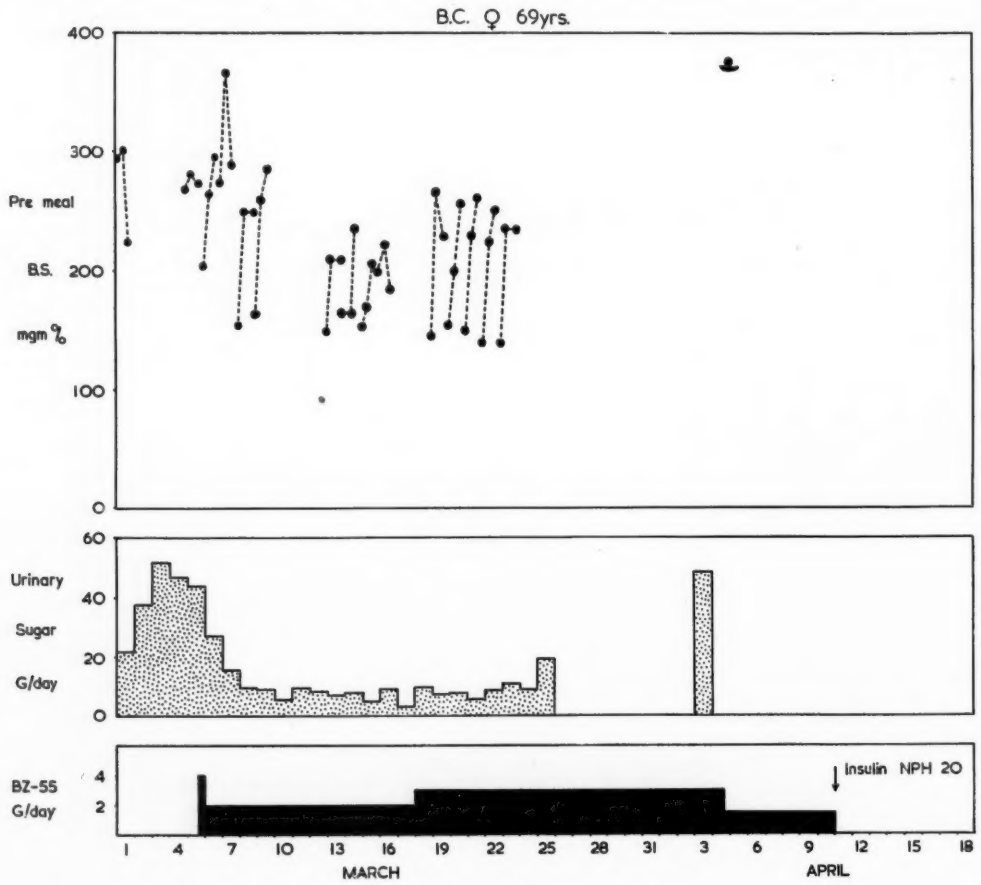


FIGURE IV
BZ–55 therapy. Case 17

difficult to reconcile this with the apparently similar effects of D 860, which possesses no antibacterial action.

In assessing the worth of an anti-diabetic substance it is desirable to compare its action with that of insulin, which is capable of lowering the level of blood sugar under all conditions. It must be effective not only in the normal animal but also in the alloxan diabetic animal,

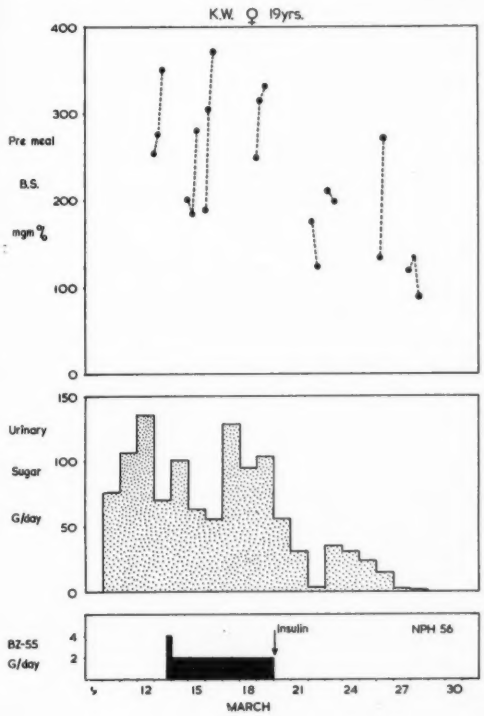


FIGURE V
BZ–55 therapy. Case 20

the pancreatectomized animal and the hepatectomized animal which is being transfused with glucose at a constant rate. Insulin increases the peripheral utilization of glucose by muscle and by brown fat. It also increases both protein anf fat synthesis by the liver. BZ-55 fails to produce any acceleration of glucose uptake by the isolated rat diaphragm, although the glucose utilization by the intrascapular brown fat body of the rat is accelerated by BZ-55 to the same degree as by insulin. A study of the effect of BZ-55 on the incorporation of radioactive acetate into the fatty acid of liver slices has shown no effect of this drug on lipogenesis.

It is therefore certain that BZ–55 cannot be regarded as an insulin substitute. How then does it control hyperglycæmia and why does this not happen in every diabetic patient? It has been shown that in the experimental animal the liver is essential for the action of BZ-55. The glucose excreted by diabetic patients arises from two sources. The first is from the ingestion of carbohydrate which fails to be utilized by the peripheral tissues, the second comes from overproduction of glucose from non-glucose sources, specifically protein, by the liver. There is reason to think that the latter is the type of diabetes of middle age—

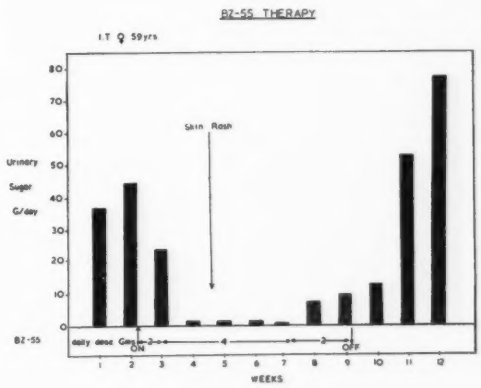


FIGURE VI
BZ–55 therapy. Case 21

the group which is most likely to show response to BZ-55. If neoglucogenesis were inhibited by the sulphonylurea drugs, this could explain their action in human beings. From recent experiments by Bornstein (1956) it would appear that inhibition of transamination is a specific action of this group of sulphonylurea drugs, and that this effect is not possessed by other sulphonamides. The reactions leading to neoglucogenesis, particularly transamination, are concerned with the synthesis of new protein and are an integral part of the entire protein balance of the body. What effect such inhibition may have if continued indefinitely is of course unknown, but it does suggest at the present that these substances should be used only with great care, and that patients under treatment should be kept under close and constant observation.

Accustomed as we are to using the absence of glycosuria as a yardstick for measuring the efficacy of our management of patients, it is a sobering thought to realize that the absence

of glycosuria in a patient treated with a sulphonylurea may prove to be a measure of harmful inhibition of the metabolism of protein by the liver.

REFERENCES

ASHWORTH, M. A., and HAIST, R. E. (1956), " Some Effects of BZ-55 (Carbutamide) on the Growth of the Islets of Langerhans ", *Canad. M.A.J.*, **74**, 975.

BERTRAM, E., BENDFELDT, E., and OTTO, H. (1956), " *Indikationen und peroralen Behandlung des Diabetes Mellitus mit einen Sulfonylharnstoffderivat* ", *Deutsche med. Wchnschr.*, **81**, 274.

BORNSTEIN, J. (1956), in the press.

Brit. M. J. (1956), **2**, 431–452, 465–467.

Canad. M. A. J. (1956), **74**, 957–998, " BZ-55 (Carbutamide) : Experimental and Clinical Studies of an Oral Antidiabetic Agent ".

DOWNIE, E., BORNSTEIN, J., HUDSON, B., and TAYLOR, K. (1956), " The Role of Sulphonylurea Derivatives in the Management of Diabetes Mellitus : A Preliminary Report ", *M. J. Australia*, **1**, 1072.

FERNER, H. (1956), " Morphological Studies of the Destruction of Alpha Cells by Chemical Means ", *Ciba Foundation Colloquia on Endocrinology*, **9**, 2. J. and A. Churchill. London.

FERNER, H., and RUNGE, W. (1956), " The Islets of Langerhans in Diabetic Patients after Treatment with Oral Antidiabetic Drug BZ-55 ", *German Medical Monthly*, **1**, 50.

FRANKE, H., and FUCHS, J. (1955), " *Ein neues antidiabetisches Prinzip : Ergebnisse Klinischer Untersuchungen* ", *Deutsche med. Wchnschr.*, **80**, 1449.

JANBON, M., LAZERGES, P., and METROPOLITANSKI, J. H. (1942), " *Étude du métabolisme du sulfa-isopropylthiodiazol chez le sujet sain et en cours de traitement. Comportement de la glycémie* ", *Montpellier méd.*, **21–22**, 489.

KIRTLEY, W. D. (1956), personal communication.

LOUBATIÈRES, A. (1944*a*), " *Analyse du mécanisme de l'action hypoglycémiante du p-amino-benzène-sulphamidothiodiazol* ", *Compt. rend. Soc. biol.*, **138**, 766.

LOUBATIÈRES, A. (1944*b*), " *Relations entre la structure moléculaire et l'activité hypoglycémiante des amino-benzènesulfamidoalkythiodiazols* ", *Compt. rend. Soc. biol.*, **138**, 830.

LOUBATIÈRES, A., BOUYARD, D. P., and FRUTEAU DE LACLOS, C. (1955), " Recent Physiological Arguments in Favour of Intrapancreatic Origin of the Hypoglycæmic and Anti-diabetic Action of p-amino-benzene-sulphamido-isopropylthiodiazol ", *Compt. rend. Soc. Biol.*, **149**, 2187.

MILLER, M., and CRAIG, J. W. (1956), " Hypoglycæmic Effects of 1-butyl-3-p-toluenesulphonylurea given Orally in Human Diabetic Subjects ", *Metabolism*, **5**, 162.

MILLER, W. L., and DULIN, W. E. (1956), " Orinase : A New Oral Hypoglycæmic Compound ", *Science*, **123**, 583.

MIRSKY, I. A., PERISUTTI, G., and DIENGOTT, D. (1956*a*), " The Inhibition of Insulinase by Hypoglycæmic Sulfonamides ", *Metabolism*, **5**, 156.

RIDOLFO, A. S., and KIRTLEY, W. R. (1956), " Clinical Experiences with Carbutamide—An Orally Given Hypoglycæmic Agent ", *J.A.M.A.*, **160**, 1285.

STOTTER, G. (1956), " Symposium on D 860 ", *Deutsche med. Wchnschr.*, **81**, 823–846.

YOUNG, F. G. (1956), " Hypoglycæmic and Anti-diabetic Sulphonamides ", *Brit. M. J.*, **2**, 431.

NORMAL VALUES FOR THE RED CELLS IN AUSTRALIA[1]

H. O. LANCASTER[2] AND NINA P. BANKS[3]

From the School of Public Health and Tropical Medicine, Sydney, and the New South Wales Red Cross Blood Transfusion Service, Sydney

SUMMARY

The means and standard deviations of the red blood cell indices of healthy Australian adults have been found to be as follows: Mean corpuscular volume (cubic micra): 89·1±2·9 for males, 87·9±6·4 for females. Mean corpuscular hæmoglobin (micromicrogrammes per cell): 33·2±1·1 for males, 32·4±2·0 for females. Mean corpuscular hæmoglobin concentration (grammes of hæmoglobin per 100 millilitres of packed cells): 37·4±1·4 for males, 37·1±1·9 for females.

The estimated mean red cell counts per cubic millimetre corresponding to the hæmoglobin values of the main survey of Walsh *et alii* (1953) are 4·73 million for males and 4·29 million for females, using the above figures for mean corpuscular hæmoglobin.

A SURVEY of the normal hæmoglobin and hæmatocrit values in an Australian population was carried out by Walsh *et alii* in 1953. As it was not practicable during that survey to include data on the properties of the red cells, we present here the results of a subsidiary survey of the red cell indices, carried out on 37 male and 63 female healthy Australian adults.

METHODS

It was decided that the most convenient source of blood would be healthy blood donors. Only new donors or those who had not given blood more than twice previously were used. None was included who had been bled during the past three months. Venous blood was obtained with a needle and syringe, after application of a tourniquet for a minimum period. Approximately five millilitres of blood were added to the dry crystals obtained by evaporating 0·1 millilitre of a solution containing 4·0 grammes of potassium oxalate and 6·0 grammes of ammonium oxalate per 100 millilitres. Duplicate estimations of the hæmoglobin and hæmatocrit values were made by techniques described by Walsh *et alii* (1953). Red cell counts were carried out with a 1 : 200 dilution of the oxalated blood in a 1% formalin 3% sodium citrate solution, the cells being counted over an area of 0·8 square millimetre. The same diluting pipette was used throughout the survey. The instruments were all properly standardized and calibrated.

[1] Received on January 31, 1957.

[2] Honorary Statistician to the New South Wales Red Cross Blood Transfusion Service.

[3] Medical Officer to the New South Wales Red Cross Blood Transfusion Service.

RESULTS

For males the mean values were as follows: hæmoglobin, 16·4 grammes *per centum*; hæmatocrit, 43·8%; and red cells 4·93 million per cubic millimetre of blood. For females the corresponding means were 14·2 grammes, 38·3% and 4·37 million per cubic millimetre.

Mean Corpuscular Volume

In Table I is given a summary of the findings on the corpuscular volume. The mean value was 89·1 cubic microns for males and 87·9 cubic microns for females, with standard deviations of 2·9 for males and 6·4 for females. These means are of the same order as those given by Wintrobe (1956) for American adults and by McLean (1938) for Australian adults. McLean gave a mean of 89 cubic microns for

TABLE I

Mean Corpuscular Volume in Australian Adults

Mean Corpuscular Volume (Cubic Microns)	Frequency of Males	Frequency of Females
75 to 77	0	1
78 to 80[1]	0	6
81 to 83	1	7
84 to 86	4	14
87 to 89	19	15
90 to 92	8	7
93 to 95	4	7
96 to 98	1	4
99 to 101	0	0
102 to 104	0	0
105 and over	0	2
Total ..	37	63

[1] 78 is to be read as 77·5 to 88·5; 78 to 80 is to be read as 77·5 to 80·5. In other words, all the figures given in this table have been rounded down.

males and 88 cubic microns for females. The extreme values recorded in our survey are 81 cubic microns and 98 cubic microns for males and 77 cubic microns and 112 cubic microns for females.

Mean Corpuscular Hæmoglobin

In Table II is given a summary of the distribution of the mean corpuscular hæmoglobin. The mean was 33·2 micromicro-

TABLE II

Mean Corpuscular Hæmoglobin in Australian Adults

Mean Hæmoglobin per Red Cell (Micromicrogrammes[1])	Frequency of Males.	Frequency of Females.
26	0	1
27	0	0
28	0	0
29	0	3
30	0	7
31	1	6
32	11	16
33	13	13
34	7	5
35	4	8
36	1	3
37	0	1
Total ..	37	63

[1] To the nearest integer.

grammes per cell for males and 32·4 micromicrogrammes per cell for females. The standard deviations were 1·1 and 2·0 respectively. These are higher than the Wintrobe (1956) figures. McLean (1938) gives 31 micromicrogrammes for males and 29 micromicrogrammes for females in his Australian series. The extreme values in our survey are 31 and 36 micromicrogrammes for males and 26 and 37 micromicrogrammes for females.

Mean Corpuscular Hæmoglobin Concentration

In Table III is given a summary of the distribution of the mean corpuscular hæmoglobin concentration. The mean was 37·4 grammes of hæmoglobin per 100 millilitres of packed cells for males and 37·1 grammes for females. The corresponding standard deviations were 1·4 grammes and 1·9 grammes. These means are higher than those of Wintrobe (1956) and those of McLean (1938). The extreme values in our survey are 34 grammes and 41 grammes for males and 29 grammes and 42 grammes for females.

DISCUSSION

It is commonly taught that the hæmoglobin concentration in the red cell is optimal and maximal. The existence of variation about the mean shows that it cannot always be maximal. We may attempt to separate out the components of variance in the indices as follows. If the coefficient of variation is small, then it approximates to the coefficient of variation of the reciprocal. It follows that the sampling coefficient of variation of any of the ratios dealt with in this paper is given by:

Coefficient of variation of $x/y =$

$$\sqrt{\{(\text{c. of v. of } x)^2+(\text{c. of v. of } y)^2\}}$$

For the hæmoglobin value, the hæmatocrit and the red cell count, the coefficients of variation may be expected to be about 0·7%, 0·5% and 2·5%. If we consider sampling errors alone, it follows that the coefficients of variation of the mean corpuscular volume, the mean corpuscular hæmoglobin and the mean corpuscular hæmoglobin concentration may be expected to be of the order of 2·6%, 2·6% and 0·8% respectively. The observed coefficients of variation for men are 3·3%, 3·2% and 3·8% and for women 7·2%, 6·3% and 5·0%. It is evident that the great bulk of the observed variation in the ratios comes from actual differences between the cells of different

TABLE III

Mean Corpuscular Hæmoglobin Concentration in Australian Adults

Mean Corpuscular Hæmoglobin Contration (Grammes[1] per 100 Millilitres of Packed Red Cells)	Frequency of Males	Frequency of Females
29	0	1
30	0	0
31	0	0
32	0	0
33	0	1
34	2	1
35	2	1
36	5	17
37	5	17
38	17	19
39	5	1
40	0	1
41	1	2
42	0	2
Total ..	37	63

[1] To nearest integer.

individuals, and that variation would still remain even if sampling error could be reduced further.

The figures for mean corpuscular volume reported in this paper are approximately the same as those quoted by Wintrobe (1956) and by Whitby and Britton (1956). On the other hand, the values for mean corpuscular hæmoglobin and mean corpuscular hæmoglobin concentration are appreciably greater. This suggests that the hæmoglobin values found in the present work are higher in relation to the

red cell count and hæmatocrit than in other series. The hæmoglobinometer used was standardized on the basis of iron assay of blood and was checked at regular intervals. It is believed to correspond with the standard value of earlier work (Walsh *et alii*, 1953).

It is significant that there have been few surveys of the standard indices reported during the past ten years. During this time the hæmoglobin equivalent of the Haldane scale has been shown to be some 6·5% higher than was previously thought. It is not surprising, therefore, that the values for the mean corpuscular hæmoglobin and the mean corpuscular hæmoglobin concentration should be appreciably greater than those reported in earlier surveys.

The mean corpuscular hæmoglobin content is calculated from the red cell count and the hæmoglobin concentration of the circulating blood. We are therefore in a position to compute the red cell count corresponding to the mean hæmoglobin value obtained in adults in the main survey of Walsh *et alii* (1953). Using the hæmoglobin values determined in that survey (15·71 grammes per 100 millilitres for males and 13·89 grammes for females) and the present figures for mean corpuscular hæmoglobin, the mean red cell count would be 4·73 million per cubic millimetre for males and 4·29 million for females.

ACKNOWLEDGEMENTS

This paper is published with the permission of the Director-General of Health, Canberra.

REFERENCES

McLEAN, J. A. (1938), "Hæmatocrit Determinations in Normal and Abnormal Blood", *M. J. Australia*, **2**, 770.

WALSH, R. J., ARNOLD, B. J., LANCASTER, H. O., COOTE, M. A., and COTTER, H. (1953), "A Study of Hæmoglobin Values in New South Wales", Special Report No. 5 of the National Health and Medical Research Council, Canberra.

WHITBY, L. E. H., and BRITTON, C. J. C. (1956), "Disorders of the Blood", Churchill, London.

WINTROBE, M. M. (1956), "Clinical Hematology", Lea and Febiger, Philadelphia.

PRIMARY SYSTEMIC AMYLOIDOSIS[1]

L. LAZARUS[2]
From St. Vincent's Hospital, Sydney

SUMMARY

A man aged forty-seven years, with no previous history of cardiac disease, suddenly presented with congestive cardiac failure, an electrocardiogram suggestive of healed infarct and a nephrotic syndrome. Hæmoptysis and hæmatemesis indicated the involvement of other systems. He died suddenly in his sleep. Autopsy showed generalized amyloid infiltration and no primary cause of amyloidosis.

The literature has been briefly reviewed. Primary systemic amyloidosis is a multisystemic disease with protean clinical manifestations. It should be considered in any patient with disease of more than one bodily system, and particularly should it be considered in any patient with cardiac failure of obscure ætiology.

THE development of systemic amyloidosis in a patient already the victim of a chronic inflammatory disease is a well known clinical entity rapidly disappearing since the advent of the antibiotics. Its occurrence in the absence of an antecedent disease is rare, but the large number of cases reported recently suggest that it is worthy of further interest.

Rokitansky is credited with first describing amyloidosis in 1842, calling it lardaceous disease of kidney, liver and spleen. The staining reaction with iodine was observed by Meckel in 1853. In 1855 Virchow noticed that the lardaceous deposits turned blue when stained with iodine and sulphuric acid. He named the deposits amyloid because of their starch-like staining property.

In the following year (1856) Wilks described a series of cases of "lardaceous disease", in two of which there was no underlying chronic disease. Wilks classified these two as "simple lardaceous disease". They are probably the first cases ever reported of primary systemic amyloidosis.

By 1946 only 46 cases had been reported (Eisen), but four years later the number had increased to 71 (Higgins and Higgins, 1950), and in 1956 Symmers was able to collect 145.

CLASSIFICATION

There have been numerous classifications of amyloidosis. The one best fitted to our knowledge of the disease at present is that of Reimann *et alii* (1935), who divide it into four types: (i) primary amyloidosis, (ii) secondary amyloidosis, (iii) tumour-forming amyloidosis, (iv) amyloidosis associated with multiple myeloma.

Secondary amyloidosis, by far the commonest variety, occurs in a patient suffering from some chronic inflammatory disease such as tuberculosis, syphilis, bronchiectasis, osteomyelitis, ulcerative colitis or rheumatoid arthritis. The organs involved are the liver, the spleen, the kidneys, the adrenals and occasionally the gastro-intestinal tract and the lymph nodes.

The term primary amyloidosis is used to designate those cases without a predisposing chronic inflammatory disease. Lubarsch, in 1929, defined criteria for diagnosis. These were as follows: (i) absence of an antecedent or coexisting disease; (ii) involvement of mesodermal tissues, such as smooth and skeletal muscle, the cardio-vascular system and the skin, rather than the liver, the spleen or the kidneys; (iii) variable staining reactions; (iv) tendency to nodular deposition. In the large number of cases reported since 1929, however, a high incidence has been demonstrated of involvement of the liver, the kidneys and the spleen; this has made classification on anatomical grounds untenable.

King (1948) proposed a classification based on distribution. He divided deposits into (i) typical (involving the liver, the spleen, the kidneys and the adrenals) and (ii) atypical (involving the heart, skeletal and smooth muscle, and the skin).

[1] Received on November 16, 1956.
[2] Medical Registrar.

In the tumour-forming type localized collections of amyloid are formed in the upper part of the respiratory tract, the conjunctiva, the bladder and the stomach. Most writers have considered these cases to be "*formes frustes*" of primary systemic amyloidosis.

It has been well known for many years that amyloidosis may occur in patients with multiple myeloma. In these cases the amyloid tends to be deposited in skeletal, cardiac and smooth muscle and in the skin.

There were numerous fine crepitations at both lung bases. Urinalysis revealed heavy albuminuria. Laboratory investigation revealed normal findings from a full blood count, from serum electrolyte and blood urea determinations, and from cytological examination of the urine. The erythrocyte sedimentation rate was 42 millimetres per hour (Wintrobe). An electrocardiogram (see Figure 1) showed sinus tachycardia, the *QS* type of *QRS* complexes in V_2 and V_3, with notching in V_3, and inverted *T* waves in leads *I* and *AVL*. This was interpreted as being due to an old anteroseptal myocardial infarct. X-ray examination of the chest showed a slightly enlarged heart with increased vascular markings.

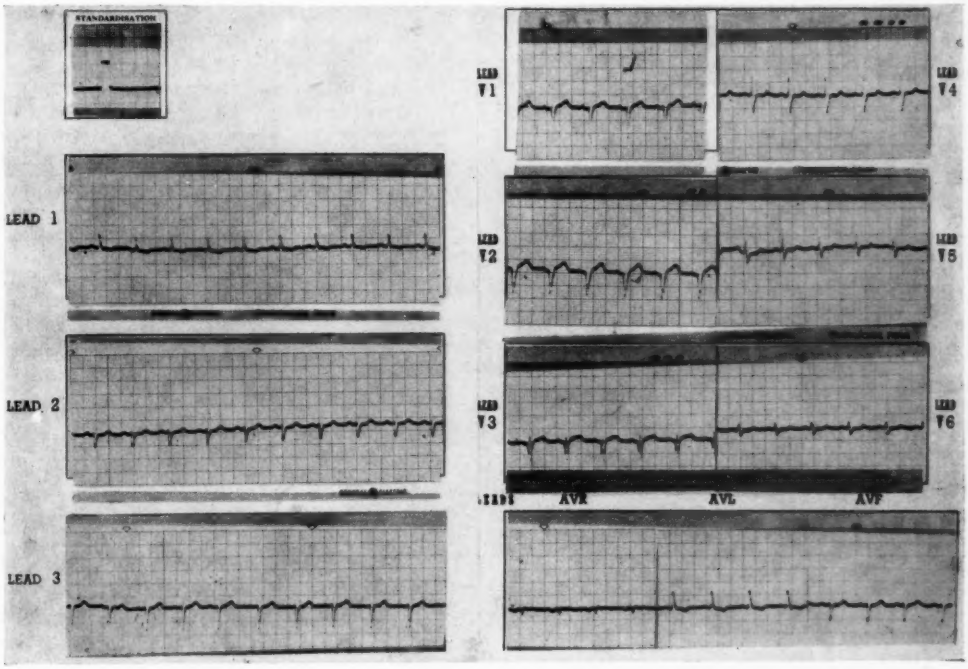


FIGURE 1
Electrocardiogram showing changes similar to those of a healed anterior infarct

REPORT OF CASE

A forty-seven-year-old male electric welder was admitted to hospital on January 26, 1956. He had been well until six months previously, when he first noticed a feeling of mild constriction in the chest related to effort and relieved by rest. For a similar period of time, he had noticed increasing generalized weakness and dyspnœa on moderate exertion. During the month prior to admission to hospital, he fainted twice after mild exertion and noticed swelling of the scrotum. He had coughed up blood-streaked sputum for three days after a "sore throat" one month prior to admission.

He was a well-built man with pitting œdema of his legs up to the mid-tibial region. His blood pressure was 100/70 millimetres of mercury. The radial pulse and jugular venous pressure were normal. The heart was not enlarged, and the heart sounds were normal.

A provisional diagnosis of congestive cardiac failure was made, and treatment was commenced with digitalis and diet of low sodium content.

The patient at first appeared to improve clinically, but œdema recurred and increased rapidly in degree. Vigorous but unsuccessful efforts were made to control his œdema with "Diamox", ion exchange resins, mercurial diuretics and intravenously administered albumin. On one occasion three weeks after admission he felt nauseated and had a small hæmatemesis. The heavy albuminuria continued throughout his illness. The specific gravity of his urine was normal at first (1020), but then consistently low readings were recorded (less than 1010). Repeated microscopic examination of urine showed only occasional hyaline casts. The blood urea content remained normal throughout. A urea concentration test showed mild diminution of function. The serum cholesterol content was 449 milligrammes per 100 millilitres.

Repeated determinations of total serum protein levels produced normal findings, but there was a gross disturbance of the albumin-globulin ratio, the serum albumin content was 1·7 grammes *per centum*, and the globulin content was 4·5 grammes *per centum*. The filter paper electrophoretic pattern showed that the γ and α2 globulins were increased in amount.

He died suddenly in his sleep on March 9, 1956, six weeks after his admission to hospital.

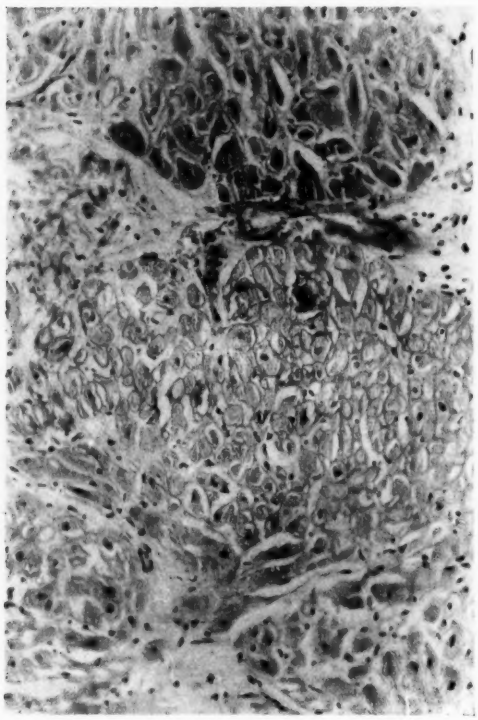


FIGURE II

Heart. Amyloid deposition around myocardial fibres and in the media of small artery. Congo red. (×120)

Autopsy Findings

Autopsy was performed ten hours after death.

Macroscopic Findings.—The macroscopic findings were as follows:

The cadaver was that of a large well developed male. Pitting œdema was present over the ankles.

The pleural cavities contained clear straw-coloured fluid, 1400 millilitres in the right, and 1000 millilitres in the left. The lungs showed no abnormality.

The heart weighed 590 grammes and showed hypertrophy and dilatation. The left ventricular wall was 21 millimetres thick, the right 6·0 millimetres thick. The freshly cut surface of the myocardium was firm and unusually pale. The valves were normal, and there was mild coronary atheroma.

The liver weighed 2230 grammes and showed "nutmeg" appearance on section.

The spleen weighed 300 grammes and was firmer than usual; the Malphigian bodies were not prominent.

The kidneys were enlarged, the right and left weighing 260 and 280 grammes respectively. The capsules stripped easily, leaving a smooth surface, and on bisection the kidney substance was pale and somewhat waxy in appearance.

No abnormalities were found in tongue, pharynx, larynx, thyroid, adrenals, œsophagus, stomach, intestines, bladder, genitalia or brain.

Microscopic Findings.—The microscopic findings were as follows:

Heart (see Figures II and III). Examination of sections stained with hæmatoxylin and eosin showed the diffuse deposition of an eosinophilic material which formed an interlacing network between the myocardial fibres. The enclosed myocardial fibres showed varying degrees of atrophy, and where atrophy was complete, there were focal deposits of the eosinophilic material. The same material was also found in the medial coats of the small coronary arteries. The abnormal material gave the typical staining reactions of amyloid with Congo red and methyl violet.

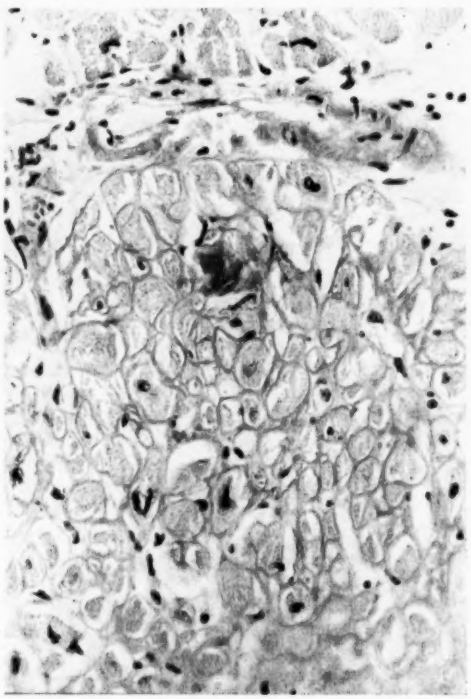


FIGURE III

Heart. High power view of same field as in Figure II. Congo red. (×240)

Kidney (see Figure IV). Amyloid was deposited in the glomeruli, arterioles and medial coats of the interlobular and arcuate arteries. The majority of glomeruli were affected.

Spleen. This showed the diffuse type of amyloid deposition.

Liver. There was moderate venous congestion. Amyloid was present only in the media of the hepatic arteries.

Lung. Amyloid was present in the media of the pulmonary arteries and was also deposited in the alveolar septa.

Stomach (see Figure V). There was diffuse deposition of amyloid in the fibres of the *muscularis mucosa*, in the media of the gastric arteries and in the supporting tissues of the gastric glands.

Marrow. Sections of the vertebral marrow showed no evidence of myelomatosis.

DISCUSSION

Amyloid is a glycoprotein with distinctive staining reactions. The protein moiety is a globulin, and the carbohydrate fraction (1% to 1·5% of the total) is a polysaccharide similar to the chrondroitin sulphuric acid in infantile cartilage (Hass, 1942).

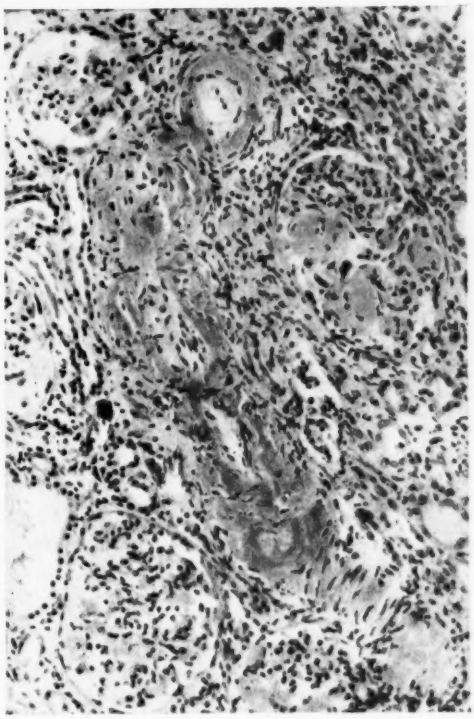


FIGURE IV

Kidney. Deposition of amyloid in glomeruli and small artery. Hæmatoxylin and eosin. (×120)

The amyloid is deposited in the intercellular spaces, particularly around muscle fibres, in the media of small arteries and beneath the endothelium of glomerular capillaries. It is a homogeneous, non-fibrillar, hyaline-like substance, which is eosinophilic, and for which certain staining methods are specific—namely, staining with Congo red, metachromasia with methyl violet and methyl green, and brown coloration with iodine. The staining reactions may be variable in cases of primary amyloidosis.

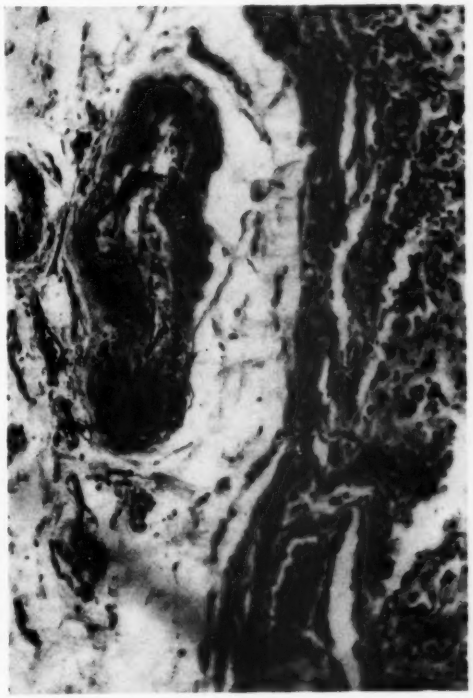


FIGURE V

Stomach. Amyloid in media of small arteries of submucosa. Congo red. (×240)

The disease is insidious in its onset and uniformly fatal in its outcome. The sexes are affected equally, and there is a predominance of patients in the older age groups (the average age is fifty-six years, according to Eisen, 1946), although cases in patients as young as nine years and as old as ninety years have been reported (Mathews, 1954). The average duration of symptoms is eighteen months (Mathews, 1954) and the longest reported duration is fourteen years (Eisen, 1946).

Cardio-vascular System

The cardio-vascular system is commonly involved (in 75% of cases, according to Mathews,

1954), and the commonest clinical picture is that of the development of intractable congestive cardiac failure in a patient with no apparent underlying cardiac disease. Often there is involvement of another system. The association of cardiac and renal disease, as in the case above, is not uncommon.

Lindsay (1946) states that cardiac symptoms and signs may result from (i) involvement of pulmonary blood vessels and alveolar walls, giving rise to *cor pulmonale*, (ii) deposition in cardiac blood vessels, resulting in coronary insufficiency, (iii) diffuse or localized interstitial infiltration of myocardium, (iv) pericardial or endocardial involvement, and (v) extensive valvular deposits sufficient to cause stenosis or insufficiency.

When the myocardium is diffusely infiltrated, the clinical picture may resemble constrictive pericarditis (Findley and Adams, 1948; Couter and Reichert, 1950). Cardiac catheterization studies (Hetzel *et alii*, 1953; Gunnar *et alii*, 1955) have revealed pressure patterns identical with those of constrictive pericarditis. This resemblance is due to the rigidity of the amyloid myocardium interfering with diastolic expansion of the heart (Gunnar *et alii*, 1955). Hypotension was recorded in 22 out of 50 cases (Mathews, 1954), and the blood pressure was raised in only three out of 22 cases reviewed by Wessler and Freedberg (1948).

The electrocardiogram has shown no typical changes in several reviews of cardiac amyloidosis (Lindsay, 1946; Josselson and Pruitt, 1953). Most cases have shown low voltage *QRS* complexes and *T* waves, auriculo-ventricular conduction defects and arrhythmias. Auricular electrical alternation has been noted (Bernreiter, 1956). In two reported cases (Wessler and Freedberg, 1948) an ante-mortem diagnosis of healed anterior myocardial infarct was made on account of the electrocardiographic changes. Abnormal electrocardiographic findings are probably due to a combination of destroyed muscle fibres and poor electrical conductivity of amyloid itself.

At autopsy the heart is usually moderately enlarged, and the amyloid deposits may be nodular or diffuse. The amyloid has a waxy appearance, and when diffusely involved, the heart wall is firm and semitranslucent. Histologically each muscle fibre is seen to be surrounded by a collar of amyloid, and some fibres are atrophied (Figure II). The rigid myocardium amply explains the loss of cardiac efficiency.

Lungs

It is uncommon for pulmonary involvement to give rise to symptoms, although in the case reported the patient had a hæmoptysis. In his review of 54 cases, in which autopsy was performed, Dahlin (1949) states that in 24 there was lung involvement. Histologically there is found to be involvement of small vessels and alveolar walls. Lesions sufficiently extensive to cause X-ray opacities and *cor pulmonale* have been reported (Sappington *et alii*, 1942). Miliary mottling of lung fields may occur.

Gastro-Intestinal Tract

The predilection for smooth muscle results in a high proportion of patients having gastro-intestinal lesions. Macroglossia is fairly common (in 12% of 50 cases reviewed by Mathews, 1954), but undue stress was placed upon its presence by earlier writers, for example Weber *et alii* (1937) and Eisen (1946); so that one would have hesitated to diagnose primary amyloidosis in its absence. Of six cases reported by Dahlin (1949), in none was macroglossia present. The enlargement is painless, and may give rise to dysarthria and dysphagia. Superficial ulceration may occur. When macroglossia occurs, biopsy may result in diagnosis. Involvement of the salivary glands causes xerostomia (Dahlin, 1949; Reimann *et alii*, 1954), and dysphagia may result from œsophageal involvement (Baber, 1947).

Hæmatemesis occurred in 6% of Eisen's cases, and in 10% of Dahlin's 54 cases. It is thought to be due to involvement of the media of small submucosal blood vessels and their subsequent rupture (see Figure V). In a case reported by Golden (1945) the patient underwent gastrectomy for hæmatemesis and pyloric stenosis. Steatorrhœa has been reported by Findley and Adams (1948). Histologically there is found to be involvement of the *muscularis mucosa*, the muscle wall and the media of small arteries.

Massive involvement of mesenteries and omenta may occur (Iverson and Morrison, 1948). There may be foci of fat necrosis in these areas, probably resulting from ischæmia.

Liver

The liver is involved in 40% to 60% of reported cases (Dahlin, 1949; Higgins and Higgins, 1950), and 10% of cases have presented primarily as liver disease (Wollaeger, 1950). Ante-mortem diagnosis of primary amyloidosis has been made in such cases by liver biopsy. The liver function tests may show evidence of

hepatic damage. Histologically the amyloid is laid down between the cells and sinusoids, and eventually replaces the cells by pressure effects.

Spleen

The spleen is involved in 40% of cases (Symmers, 1956) and may show focal or diffuse infiltration identical with that seen in classical cases of secondary amyloidosis.

Kidneys

Involvement of the kidneys is probably the commonest lesion in secondary amyloidosis. Until recently it was considered very rare in primary amyloidosis. However, Lindsay (1948) and Mathews (1954) reported that in 40% to 50% of cases there was evidence of kidney lesions. Muehrcke *et alii* (1955) reviewed nine cases with nephrotic syndrome; in six of these the patient died in uræmia and in five with hypertension. The average period of life after appearance of œdema was sixteen months.

Muehrcke followed one patient by means of serial renal biopsies over a period of twelve months, from pure nephrosis to scarred kidney with azotæmia.

Skin.

Deposits in the skin are common, occurring in 33% of cases (Higgins and Higgins, 1950). Goltz (1952) has extensively reviewed the literature regarding skin lesions. Purpura is the commonest lesion. It is most predominant in body folds and is due to involvement of skin vessels by amyloid. The lesions often result from minor trauma, and the Hess test may show increased capillary fragility. Waxy translucent papules and plaques occur most commonly on the eyelids and the naso-labial folds and in the inguinal and perianal regions. They commonly occur in groups and may appear on the buccal mucous membrane. Subcutaneous nodules of varying size may occur anywhere. A diffuse involvement of skin may occur and is commonest on head and neck. Also described are alopecia and brittleness of nails.

Nervous System

There may be peripheral neurological signs due to ischæmic neuropathy secondary to involvement of the *vasa nervorum* (Kernohan and Woltman, 1942). There are no reported cases of involvement of the central nervous system.

Miscellaneous

Lymphadenopathy has been observed in 25% of cases and is of diagnostic import. Arthritis may be simulated, and involvement of bones, joints and tendons may lead to limitation of movement, disturbance of gait and pathological fracture (Koletsky and Stecher, 1939). The endocrine glands may be involved, although functional disturbances are rare. The adrenals may be as severely affected as in secondary amyloidosis. Amyloid goitre has been noted (Walker, 1942). Fatigue and pains in extremities may be due to involvement of striated muscle. A moderate hypochromic anæmia is common, and the erythrocyte sedimentation rate is frequently raised. Hyperglobulinæmia is not uncommonly observed (Symmers, 1956). The γ globulins are commonly raised, and there may be a moderate or large increase in the $\alpha 2$ and β globulins.

Ætiology

The precise ætiology of amyloidosis is unknown, but there is much evidence of an experimental nature suggesting a relationship between amyloidosis and hyperglobulinæmia. The well known occurrence of amyloidosis in multiple myeloma also indicates a possible relationship to altered serum globulins. The amyloidosis in multiple myeloma resembles primary amyloidosis in age incidence, organ distribution and staining variability.

Magnus-Levy (1938) considered that Bence-Jones protein was chemically related to amyloid. This is an interesting observation, as nearly all patients with multiple myeloma who develop amyloidosis have Bence-Jones proteinuria (10 out of 11 cases reviewed by Snapper *et alii*, 1953).

It has been noted, however, that hyperglobulinæmia is uncommon in myeloma patients with amyloidosis. Eisen (1946) states that several cases have been reported in which hyperglobulinæmia was noted before the appearance of amyloid, after which the serum globulin level fell. Apitz (1940) suggested that this was due to depositions of serum globulins as amyloid and stressed that any patient with primary amyloidosis should be carefully examined for multiple myeloma. He even went so far as to say that all cases of primary amyloidosis were due to multiple myelomatosis.

On the experimental side it has been noticed that horses used for production of immune sera develop amyloidosis. Reimann and Ekland (1935) produced amyloidosis in rabbits by injecting them with sodium caseinate for eight to thirteen months. Other workers have produced amyloidosis in animals by injections of pus, vaccines, human sera and egg albumin and by dietary measures. In all these instances hyperglobulinæmia has developed.

In most cases of primary amyloidosis, however, the patients have normal serum proteins and the relationship of the condition with experimentally produced amyloidosis is obscure. The causal factor is at present unknown; and until such time as it is discovered, our present classification as primary must remain.

Diagnosis

The ante-mortem diagnosis may be established by biopsy or Congo red test.

Biopsy should always be performed when there is a suitable lesion—for example, a skin nodule, hepatomegaly, lymphadenopathy or macroglossia. Gum biopsy was popularized by Selikoff and Robitzek (1947), who found amyloid in 14 out of 47 cases of secondary amyloidosis. The positive results recorded in cases of primary amyloidosis have been much fewer in number. Symmers (1956) states that gum biopsies are usually very small, and that the surgical trauma involved in obtaining the specimen makes them unsuitable for histological study.

The Congo red test has variable results in primary amyloidosis. Jackson and Slavin (1954) claim that the result of the test is positive only if there is extensive liver involvement, as other tissues do not constitute sufficient bulk to absorb the dye. A review of cases in which positive results were obtained in Congo red tests lends support to this theory.

Of 50 cases (Mathews, 1954) 12 were diagnosed *ante mortem*, seven by biopsy, three on clinical grounds, and two by Congo red. Snapper *et alii* (1953) suggest injection of Congo red for establishing the diagnosis in skin nodules, which will turn a "fiery red colour". This attitude is to be deplored, as the discoloration is permanent.

Prognosis

There are no reported cases of recovery from primary amyloidosis. The average survival period after the appearance of first symptoms in Eisen's series was thirty-two months. The longest surviving patient lived for fourteen years.

Treatment

There is no known treatment. On the basis of a possible hyperimmune ætiology several earlier writers suggested the use of ACTH and cortisone. These have been used in several cases with no beneficial effect.

Acknowledgements

I am grateful to Dr. R. Jeremy and Dr. B. Hall for permission to publish this case, and to Dr. P. K. Lamond for the autopsy report.

References

Apitz, K. (1940), "*Die Paraproteinosen*", *Arch. path. Anat.*, **306**, 631.

Baber, M. (1947), "Amyloid Macroglossia", *Lancet*, **1**, 210.

Bernreiter, M. (1956), "Unusual Electrocardiographic Findings in Cardiac Amyloidosis", *J.A.M.A.*, **161**, 441.

Couter, W. T., and Reichert, R. E. (1950), "Primary Systemic Amyloid Mimicking Chronic Constrictive Pericardial Disease", *Circulation*, **2**, 441.

Dahlin, D. C. (1949), "Primary Amyloidosis with Report of Six Cases", *Am. J. Med.*, **1**, 144.

Eisen, H. (1946), "Primary Systemic Amyloidosis", *Am. J. Med.*, **1**, 144.

Findley, J., and Adams, W. (1948), "Primary Systemic Amyloidosis Simulating Constrictive Pericarditis", *Arch. Int. Med.*, **81**, 342.

Golden, A. (1945), "Primary Systemic Amyloidosis of the Alimentary Tract", *Arch. Int. Med.*, **75**, 413.

Goltz, R. W. (1952), "Systematised Amyloidosis: A Review of the Skin and Mucous Membrane Lesions and a Report of Two Cases", *Medicine*, **31**, 381.

Gunnar, R. M., Dillon, R. F., Wallyn, R. J., and Elisberg, E. I. (1955), "The Physiologic and Clinical Similarity between Primary Amyloid of the Heart and Constrictive Pericarditis", *Circulation*, **12**, 827.

Hass, G. (1942), "Studies of Amyloid: II. The Isolation of a Polysaccharide from Amyloid Containing Tissue", *Arch. Path.*, **34**, 92.

Hetzel, P. S., Wood, E. H., and Burchell, H. B. (1953), "Pressure Pulses in the Right Side of the Heart in a Case of Amyloid Disease and in a Case of Idiopathic Heart Failure Simulating Constrictive Pericarditis", *Proc. Staff Meet. Mayo Clinic*, **28**, 107.

Higgins, W. H., and Higgins, W. M. (1950), "Primary Amyloidosis", *Am. J. M. Sc.*, **220**, 610.

Iverson, L., and Morrison, A. B. (1948), "Primary Systemic Amyloidosis", *Arch. Path.*, **45**, 1.

Jackson, A., and Slavin, M. (1954), "Primary Amyloidosis", *Am. Heart J.*, **47**, 839.

Josselson, A., and Pruitt, R. (1953), "Electrocardiographic Findings in Cardiac Amyloidosis", *Circulation*, **7**, 200.

Kernohan, J. W., and Woltman, H. W. (1942), "Amyloid Neuritis", *Arch. Neurol. & Psychiat.*, **47**, 132.

King, L. S. (1948), "Atypical Amyloid Disease, with Observations on a New Silver Stain for Amyloid", *Am. J. Path.*, **24**, 1095.

Koletsky, S., and Stecher, R. M. (1939), "Primary Systemic Amyloidosis—Involvement of Cardiac Valves, Joints and Bones with Pathologic Fracture of the Femur", *Arch. Path.*, **27**, 267.

Lindsay, S. (1946), "The Heart in Primary Systemic Amyloidosis", *Am. Heart J.*, **32**, 419.

Lubarsch, O. (1929), "*Zur Kenntnis ungewöhnlicher Amyloidablablagerungen*", *Arch. path. Anat.*, **271**, 867.

Magnus-Levy, A. (1938), "Multiple Myeloma", *Acta. med. scandinav.*, **95**, 218.

Mat iews, W. H. (1954), "Primary Systemic Amyloidosis", *Am. J. M. Sc.*, **228**, 317.

Meckel, J. F. (1853), *Annalen des Charite Krankenhaus zu Berlin*, **4**, 264.

MUEHRCKE, R. C., PIRANI, C. L., POLLACK, V. E., and KARK, R. M. (1955), "Primary Renal Amyloidosis with the Nephrotic Syndrome Studied by Serial Biopsies of the Kidney", *Guy's Hosp. Rep.*, **104**, 295.

REIMANN, H. A., KOUCKY, R., and EKLUND, C. M. (1935), "Primary Amyloidosis Limited to Tissue of Mesodermal Origin", *Am. J. Path.*, **11**, 977.

REIMANN, H. A., SAHYOUN, P. F., and CHAGLASSIAN, M. T. (1954), "Primary Amyloidosis", *Arch. Int. Med.*, **93**, 673.

REIMANN, H. A., and EKLUND, C. M. (1935), "Long Continued Vaccine Therapy as a Cause of Amyloidosis', *Am. J. M. Sc.*, **190**, 88.

ROKITANSKY, K. (1842), "*Handbuch der Pathologischen Anatomie*", **3**, 311.

SAPPINGTON, W. S., DAVIE, J. H., and HARNOFF, J. A. (1942), "Primary Amyloidosis of the Lungs", *J. Lab. & Clin. Med.*, **27**, 882.

SELIKOFF, I. J., and ROBITZEK, E. H. (1947), "Gingival Biopsy for the Diagnosis of Generalised Amyloidosis", *Am. J. Path.*, **23**, 1099.

SNAPPER, I., TURNER, L. B., and MUSCOVITZ, H. L. (1953), "Multiple Myeloma", Grune and Stratton, New York.

SYMMERS, W. St. C. (1956), "Primary Amyloidosis: A Review", *J. Clin. Path.*, **9**, 187.

VIRCHOW, R. (1855), "*Ueber den Ganz der Amyloiden Degeneration*", *Arch. path. Ant.*, **8**, 364.

WALKER, G. A. (1942), "Amyloid Goitre", *Surg., Gynec. & Obst.*, **75**, 374.

WEBER, F. P., CADE, S., STOTT, A. W., and PULVERTAFT, R. J. V. (1937), "Systematised Atypical Amyloidosis with Macroglossia", *Quart. J. Med.*, **6**, 181.

WESSLER, S., and FREEDBERG, A. S. (1948), "Cardiac Amyloidosis", *Arch. Int. Med.*, **32**, 63.

WILKS, S. (1856), "Cases of Lardaceous Disease and Some Allied Affections", *Guy's Hosp. Rep.*, **2**, 105.

WOLLAEGER, E. (1950), "Primary Systemic Amyloidosis with Symptoms and Signs of Liver Disease: Diagnosis by Liver Biopsy", *M. Clin. North America*, **34**, 1113.

THE HISTOLOGY OF GENERALIZED PULMONARY EMPHYSEMA

I. THE GENESIS OF THE EARLY CENTROLOBULAR LESION: FOCAL EMPHYSEMA[1]

K. H. McLEAN[2]

From the Department of Pathology, University of Melbourne

SUMMARY

The histological appearances of structural changes in air passages in the early lesions of generalized pulmonary emphysema are presented. The earliest lesions were found in respiratory bronchioles and alveolar ducts near the middle of secondary lobules; one form was an acute lesion associated with bronchiolar obstruction proximally, whereas the other was " chronic ", with no evidence of recent change or obstruction.

The chronic form evolved either towards diffuse emphysema or towards macroscopically visible focal emphysema in which sharply localized dilated air spaces occupy the middle of the lobule.

An hypothesis of origin of these early subclinical lesions is presented. It rests on two propositions: first, that distending forces exist in patent air passages beyond an obstruction (particularly in those just distal to such an obstruction) and that these forces have a disruptive effect; second, that obliteration of bronchioles, due to past inflammation, occurs diffusely and extensively in emphysema, even in these early examples. Both phenomena have been shown to be closely related to acute bronchiolitis (McLean, 1956*b*, 1957) so that the disease is regarded as a sequel of repeated incidents of acute bronchiolitis, progressing more rapidly when such incidents are severe or prolonged.

This hypothesis is consistent not only with pathological observations on the emphysematous lung but also with morphological, clinical and physiological observations concerning the broader subject of the evolution of inflammatory disease of the bronchioles.

Concurrently, the nature of the black pigment of the lung and its relation to focal emphysema were examined. It was concluded that, in most cases, part of this pigment is endogenous hæmosiderin derived largely from the hæmoglobin of cells found in the exudate in acutely inflamed respiratory bronchioles and branches. Evidence that part is carbon rests on less conclusive evidence, although it is not denied that inhaled carbon can contribute to this pigmentation. Excessive retention of carbon or hæmosiderin in the peripheral passages was considered to result in accentuation of the focal form of emphysema.

> Let us try to analyze the plot of this story or drama which repeats itself in such different contexts and in such various forms.
>
> A. J. TOYNBEE, " The Study of History ", Vol. I, Part II, C (ii) (*b*) i.

MOST descriptions of the histology of pulmonary emphysema have been concerned with the extensive structural changes accompanying the clinical disease. Morphologically, this is a late stage, and attempts to recognize the factors producing the lesions have been liable to the same pitfalls of logic that beset the archæologist. However, whereas the archæologist is obliged to synthesize hypotheses from observations on end results, the morphologist, if he cares to disinter them, is provided with a complete sequence of changes from the earliest to the most advanced lesions. The continuity of this sequence having been demonstrated, early changes can then be studied and the primary phenomena recognized.

In a presentation of the macroscopic morphology of pulmonary emphysema it was shown that the earliest macroscopically visible lesion (from which more advanced forms evolved) was seen as slight dilatation of the air passages in the middle of the secondary lobule (McLean, 1956*a*) The histological changes in the air passages illustrating the evolution and variants of this early centrolobular or " focal " lesion are presented here. Such early lesions long precede the development of clinical symptoms due to reduced pulmonary function and do not show many of the secondary changes found in more advanced disease. Indeed, in the late stages all evidence of the mode of develop-

[1] Received on August 27, 1956.

[2] Research Fellow, Department of Pathology, University of Melbourne.

ment of the condition has vanished, and it was only in these early changes that sufficient evidence was available to permit the promulgation of a theory of pathogenesis.

MATERIAL AND METHODS

The material and methods have been described in detail previously (McLean, 1956*b*). Of the selected blocks of tissue that were used in this earlier investigation, most showed some evidence of emphysema, particularly in its early forms. These blocks, together with five others from emphysematous lungs that were cut subsequently, were serially sectioned. The stain for iron used here was the Prussian blue method of Gömöri (1936).

THE AIR PASSAGES

"Normal" Lung

When blocks were cut from either macroscopically normal or emphysematous lungs, an attempt was made, whenever possible, to recognize and include in the block at least most of one secondary lobule—the smallest unit of lung tissue enclosed by connective tissue septa (Figure I). In some blocks of macroscopically normal lungs no histological evidence of emphysema was found. In these, except on the borders of larger units such as subsegments or segments, the interlobular septa were thin; enclosed in the septa were tributaries of the pulmonary veins and, on one side of the lobule, generally remote from the larger veins, a bronchiole and its attendant pulmonary arteriole were found; from both of these a branch entered the lobule.

The bronchiole entering the lobule divided five or six times to end in a number of terminal bronchioles, all of which were found nearer the middle of the lobule than the periphery (Figure I). From this inner zone branches of terminal bronchioles led towards the periphery and to that central part of the lobule not occupied by bronchiolar and arteriolar divisions.

Reconstruction of serial sections was essential in order to recognize the passages distal to the terminal bronchiole, even its immediate divisions—the respiratory bronchioles. Of these, three orders were usual, the first having an almost complete wall, whereas in those of the third order epithelium was seen only on one side of the lumen, usually that nearest the accompanying arteriole. The remainder of the wall consisted of an evenly perforated, interlacing mesh of musculo-elastic tissue continuous with that beneath the epithelialized part.

In their next division—the alveolar ducts—the whole wall was composed of such a mesh, the bundles of which were usually more delicate. Beyond these ducts the mesh outlining further passages contained little or no muscle; recent writers have described these passages as further alveolar ducts (von Hayek, 1952) or as atria (Heppleston, 1954*b*). Since the distinction between these passages and ducts cannot be effected without meticulous reconstruction, they will subsequently be described generically as ducts. These ducts then terminated in alveolar sacs.

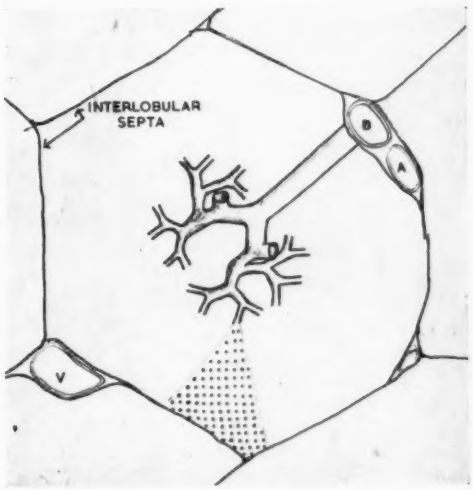


FIGURE I

Diagrammatic representation of bronchiolar divisions within a secondary lobule; for clarity only half of the smaller divisions have been included. The approximate distribution of one terminal bronchiole is shaded. Note that the terminal bronchioles (and their immediate branches) are situated in the middle of the lobule. B: bronchiole: A: artery; V: vein

From respiratory bronchioles to alveolar sacs, each perforation in the mesh led to an alveolus, each of which was discrete and separate and was in effect a diverticulum of the main passage. Unless they abutted on bronchioles, arterioles or septa, the outer parts of the alveolar walls were contiguous with those of adjacent passages.

Thus in single sections examined at low magnification the main passage, for instance, an alveolar duct, was outlined by the mesh cut in section, the area being enclosed by the walls of the surrounding layer of alveoli (Figure II).

In these macroscopically normal lungs, showing no dilatation of air passages, some evidence of old inflammatory change was often found, particularly in the small bronchioles within the lobules. Alveolar walls connected

each strand of the mesh to the adjoining strands, and in such sections of normal lung, isolated strands of elastic tissue were never seen. In these bronchioles, apparently occurring at random, occasional areas of their walls showed loss of smooth muscle and irregularities in the subepithelial elastic net; such changes were

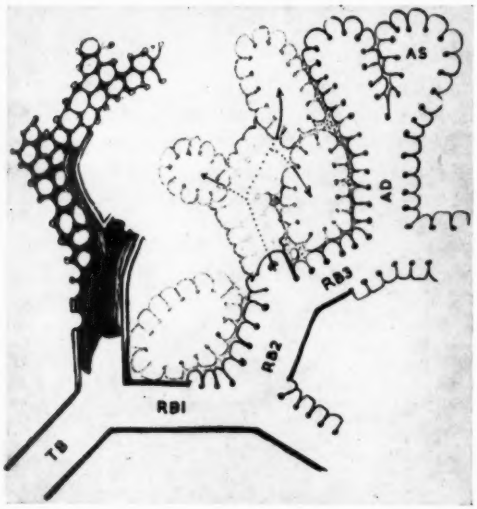


FIGURE II

Diagrammatic representation of part of the ramifications of a terminal bronchiole (TB): three orders of respiratory bronchioles (RB_1, RB_2 and RB_3), alveolar ducts (AD) and finally alveolar sacs (AS). Part is illustrated two-dimensionally (right) to emphasize that alveoli are diverticula of these passages. The musculo-elastic mesh is depicted alone (left) in three-dimensional form, cut longitudinally

most obvious in terminal and first order respiratory bronchioles. Less often, secondary communications between bronchioles and adjacent air spaces (discussed previously—McLean, 1957) were seen, particularly in terminal and pre-terminal bronchioles. Old obliterative lesions of inflammatory origin were, however, rarely recognized. Partial occlusion was uncommon, and complete bronchiolar obliteration was never identified in a lobule in which there was no histological evidence of emphysema. In some cases, scattered recent occlusions with mucus or inflammatory exudate were present without change in the aerated passages beyond the plugs.

Acute Focal Emphysema

In some of the material examined it was possible to conclude that excessive dilatation of air spaces was a recent phenomenon. The best criterion of age was the form and degree of inflammatory and exudative changes.

In one case most of the lung was "normal" with no histological evidence of emphysema and insignificant old inflammatory damage. However, in some areas there was extensive obstruction by mucus; some passages supplying whole groups of secondary lobules had had their arborizations completely occluded down to the second or third order bronchioles. Aeration of most such lobules had been maintained by collateral ventilation, and, in these, the peripheral air passages appeared normal. However, the air passages just distal to plugs (usually alveolar ducts in this case) were often somewhat dilated, and in most of these passages alveoli in their walls were filled with mucus-laden macrophages, so that the passage had the appearance of a simple tube (Figure III). The degree of dilatation varied, but was most obvious where air had had to traverse several secondary lobules to reach the affected area.

Similar changes were seen in acute bronchiolitis in which there was minimal inflammatory involvement of passages beyond the respiratory

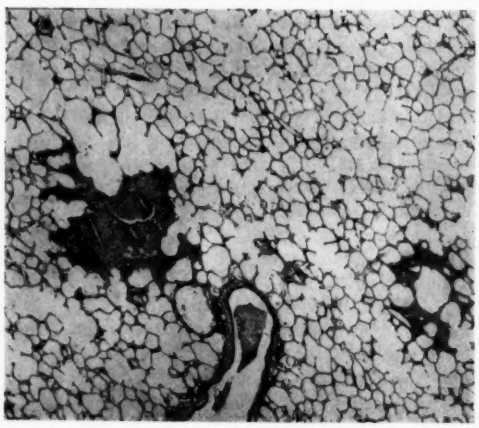


FIGURE III

Photomicrograph showing dilated air passages immediately peripheral to exudate in and around respiratory bronchioles. In adjacent serial sections this exudate completely occluded the terminal and respiratory bronchioles. (Dilatation is increased somewhat by shrinkage of exudate). (×16)

bronchioles. Plugs of exudate extended to second or third order respiratory bronchioles, beyond which the passages were aerated and often dilated. Of these the most clear-cut examples of recent emphysema were also associated with material filling the alveoli in

the wall of the passage, the material in these cases being cellular exudate rather than macrophages.

This form of acute emphysema has been termed "focal", since dilatation, although only visible microscopically, was observed in small foci near the branches of terminal bronchioles with no change in passages on the periphery of secondary lobules.

Chronic Focal Emphysema

A more common and often more advanced form of dilatation of air spaces in the middle of secondary lobules was that seen in the absence of recent obstructive or inflammatory changes. The dilatation and the associated changes were then assumed to be older lesions than those just described; how much older could not be determined.

As a general rule, in the lungs of young people this change was visible only histologically but, when present, was usually widespread. In older patients the dilated spaces became more obvious and were found in an increasingly higher proportion, so that generalized focal emphysema was visible macroscopically in most males beyond middle age (McLean, 1956*a*).

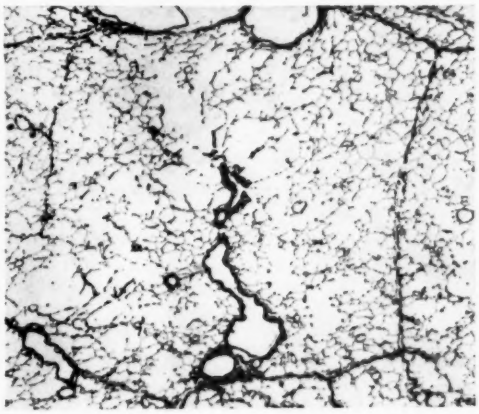


FIGURE IV

Photomicrograph showing most of a secondary lobule with early focal emphysema. Centrally, a "terminal bronchiole" opens into a "common pool". Respiratory bronchioles could not be recognized. Relatively normal peripheral passages arise from the common pool. Emphysema was not visible macroscopically. (×6)

Thus the lesions can be divided conveniently into those recognizable only in sections and those visible macroscopically.

Microscopic Emphysema.—Pathological dilatation of air spaces was often found in sections cut from macroscopically normal lung. In the earliest lesions in which this dilatation could be recognized the air passages near the periphery of secondary lobules were normal. Different passages were affected but most frequently they were the two distal orders of respiratory bronchioles or the first order of alveolar ducts.

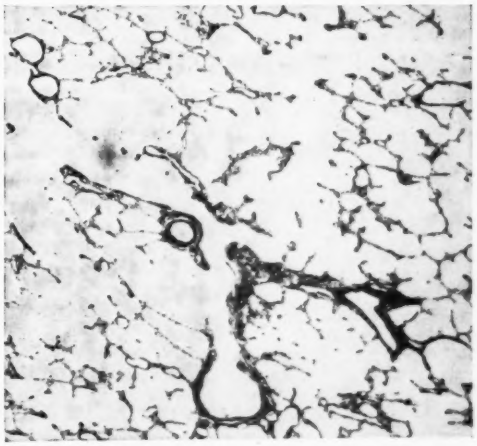


FIGURE V

Photomicrograph showing disruption of the alveolar walls in respiratory bronchioles, leaving bare musculo-elastic strands (above). Branches of one respiratory bronchiole (upper left) communicate freely with a defect in the wall of a more proximal passage (below). The precise identification of the terminal bronchiole and its divisions is clearly impracticable even at this early stage. (×22)

Significant dilatation of the main passage outlined by the musculo-elastic mesh was always associated with breakdown of the walls of the alveoli of the affected passage, although some often remained intact—usually on the side near the adjacent arteriole. Thus free communications developed between adjacent passages, leaving behind bared strands of musculo-elastic tissue, which, in sections, appeared as isolated dots—structures not seen in normal lungs (Figures IV and V).

In slightly more advanced examples the process affected all the first four divisions of the terminal bronchiole (although all divisions were not affected to the same extent), so that the terminal bronchiole became surrounded by a maze of dilated and intercommunicating passages, many of which were traversed by musculo-elastic strands (Figure IV). Hence even in this minimal lesion the original passages

often could not be recognized, and their only remnants were disordered strands of tissue.

Thus branches of terminal bronchioles opened into what will be referred to subsequently as " common pools ", from which passages to the unaffected periphery arose. As the size of these " common pools " around the terminal

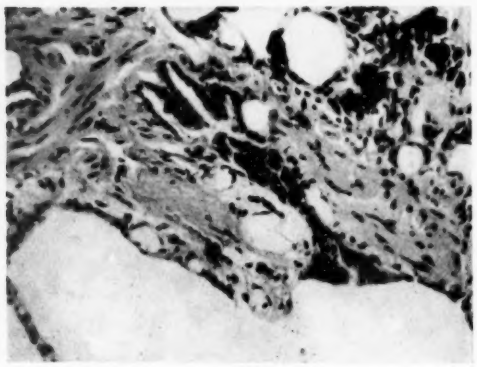


FIGURE VI

Photomicrograph showing pigment-filled macrophages occluding the lumen of a bronchiole opening into a " common pool " in early focal emphysema. There is a relatively large amount of smooth muscle in the region. Hæmatoxylin and eosin. (×120)

bronchioles increased, adjacent ones became confluent, so that most of the middle of a lobule was occupied by dilated air spaces communicating freely with one another. At this stage the lesion became apparent macroscopically. Concurrently with increasing size of these spaces, changes became evident in the passages leading to them. Mainly because of confusion between what may have been secondary communications in the walls of terminal (and even pre-terminal) bronchioles and breakdown of alveoli in the walls of respiratory bronchioles, identification of the original terminal bronchiole was often in doubt (Figure V). As a result the original terminal bronchiole may frequently have been labelled " respiratory ". The reverse phenomenon also occurred, and respiratory bronchioles may have been labelled " terminal ", owing to filling of the alveoli in their walls by organized exudate or pigment-filled macrophages. Since the original terminal bronchiole usually could not be indisputably recognized, orders of respiratory bronchioles could not be named.

Especially at the stage where the " common pool " had developed, the original terminal bronchiole could never be identified, and structures having the characteristics of respiratory bronchioles were never present. Thus the use of these terms, which imply a recognition of original structures, will be avoided in describing more advanced lesions.

Typically, a short arborization of fully epithelialized passages opened, often through simple defects in their walls, directly and indiscriminately into the " common pool ". Distal to these openings the epithelium sometimes continued a variable distance on the wall of the " pool ", usually overlying irregular aggregations of musculo-elastic tissue or fibrous connective tissue, which were often closely applied to the walls of arteries.

When a bronchiole supplying a lobule was traced peripherally to its most proximal communications with the common pools, no more than four divisions were ever recognized; often only three were present. Each of these divisions showed evidence of damage due to past inflammation, most change being distal. In early cases these changes were restricted to the last two or three divisions and included diminution of muscle (occasionally only slight),

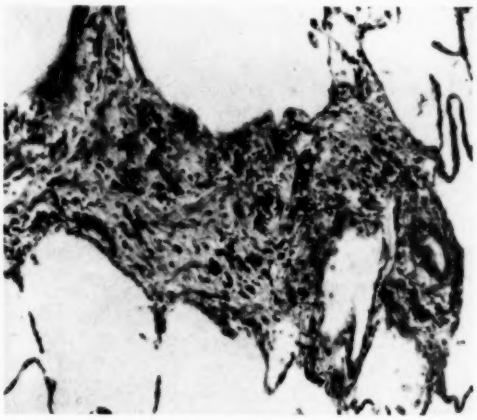


FIGURE VII

Photomicrograph showing remnants of an obliterated division of a medium-sized bronchiole (part of which is seen at upper left), from a patient with prolonged asthma and slight macroscopic emphysema. A small amount of elastic tissue remains around a group of epithelial cells (right)—elsewhere in the scar there are only occasional fibres. Elastin stain. (×90)

distortion of the subepithelial elastic net and, very often, formation of a thin layer of vascular connective tissue between the net and the epithelium—that is, partial organization and obliteration of the lumen (McLean, 1956*b*).

Complete obliterative changes were rarely found. Obvious recent occlusion by pigment-filled macrophages was seen occasionally

FIGURE VIII

Photomicrograph of a section of coal-miner's lung showing a bronchiole opening into two dilated spaces, the walls of which contain much black pigment. More peripheral passages (arising from the two "common pools") are relatively normal. (×16)

(Figure VI), but clear examples of permanently obliterated bronchioles were not recognized except where several divisions were involved (Figure VII). The epithelium lining passages

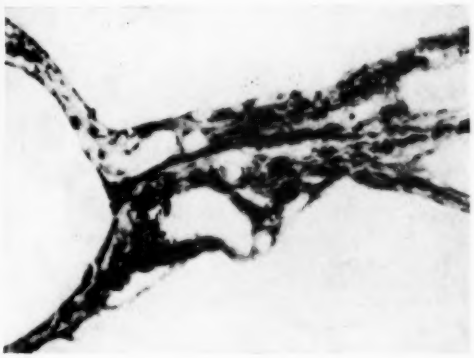


FIGURE IX

Photomicrograph showing residual tissue in an area of focal emphysema. One space (left) is partly lined by cuboidal epithelium continuous with a narrow cord of cells deep in the tissue. This epithelium was not continuous with that lining recognizable bronchioles. (×160)

entering the "pool" was often continuous, by a narrow cord of epithelial cells, with epithelium lining small areas of their outer walls. Such formations were often numerous, and although they emphasized the considerable structural changes, it was not clear whether they represented obliterated passages, not to mention whether such passages were original airways or secondary communications.

Macroscopic Focal Emphysema.—On macroscopic examination, at the stage where dilated spaces in the middle of the lobule were just visible, the periphery appeared normal, and in most cases this was confirmed histologically. However, in a few examples even at this stage the process of dilatation of passages and breakdown of their walls was seen extending out towards the periphery. In all these cases there was little fibrosis or pigment distal to the bronchioles. With this early diffuse extension

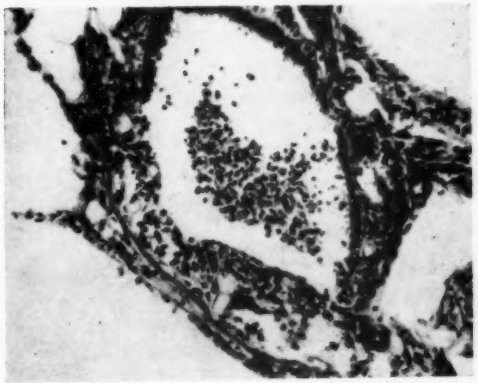


FIGURE X

Photomicrograph showing, in a region of macroscopic focal emphysema, a bronchiole two divisions beyond a terminal bronchus. There was little muscle remaining other than that illustrated (left). The epithelium is flat and attenuated in part, and the lumen contains polymorphonuclear cells and macrophages. There is much black pigment in the wall. (×60)

of the "common pool" the centrolobular pattern became obscured, and the lesions could no longer be described as "focal".

More commonly the central spaces continued to increase in size without significant change in the peripheral passages, so that the focal lesions became increasingly more obvious (Figure XI). Such lesions were typical of macroscopic focal emphysema. Both macroscopically and in sections it was observed that there were often excessive amounts of black pigment in the "focus". On occasions, deposition of pigment was extreme, as, for instance, in the lungs of two coal miners; but usually there was only a moderate quantity.

However, in some examples little pigment was found, but in such cases the walls of the dilated spaces were often relatively thick and collagenous. In only a few lungs showing macroscopic focal lesions were there insignificant amounts of pigment or collagen in a " focus " ; such lobules generally showed some diffuse change on the periphery of the lobule. (More detailed consideration of black pigment is presented below.)

respiratory bronchioles was found, and all that could be concluded from such reconstructions was that the spaces forming the foci apparently represented the remnants of all orders of respiratory bronchioles together with many peripheral alveolar ducts and all ducts and sacs in the middle of the lobule. Since the original terminal bronchiole could not be identified, the proximal limit of the passages involved could not be determined.

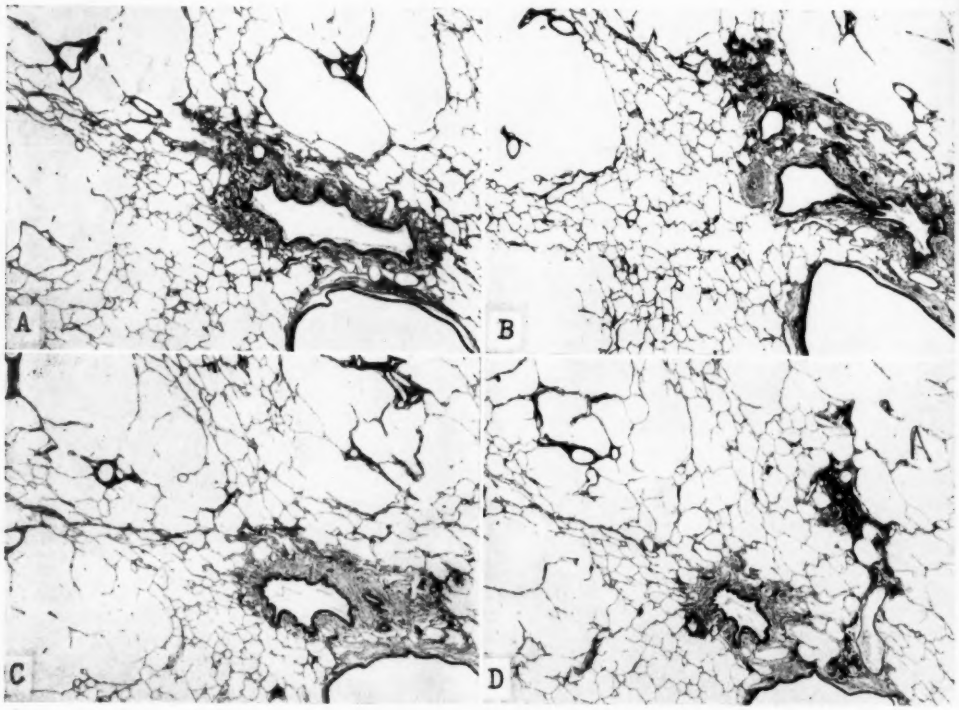


FIGURE XI

Four photomicrographs of sections at approximately 200μ intervals of a lung with macroscopic focal emphysema showing the relations of an occluded terminal bronchus. The large diverticulum of a terminal bronchus (right, B) ends blindly in scar tissue (C). The scar tissue, containing much pigment, extending peripherally (D), in relation to an arterial branch. Where the scar ends (upper right, D) the spaces were seen, with higher magnification, to be lined by epithelium. Apart from this obliterated large bronchiole there are also numerous small diverticula (C, D) and scar tissue (A, B). (×12)

The common pool in macroscopic focal emphysema was often divided into two or sometimes more parts with only somewhat indirect communications. Particularly in those examples with gross deposition of pigment in the foci, even branches of " terminal " bronchioles led to discrete groups of spaces. Three-dimensional reconstruction of these dilated spaces was largely uninformative ; no structure having any of the features of

The walls of the dilated spaces were often irregularly thickened, sometimes outstandingly so where deposition of black pigment was great (Figure VIII). In most cases accumulations of connective tissue, usually containing pigment, were most obvious around arteriolar branches, but occasionally they were found remote from both the bronchiole entering the space and the larger arteriolar branches. All such masses sometimes contained irregular elastic tissue

fibres and, less often, contained muscle cells, but these specialized tissues were sparse and in no case aided significantly the interpretation of reconstructions.

In most lesions bronchioles opened directly into the dilated spaces (Figure XII), the epithelium rarely extending on to the wall of

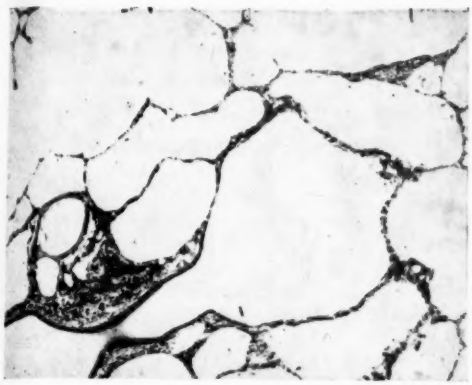


FIGURE XII

Photomicrograph showing a bronchiole ending abruptly by opening into a dilated space (which communicated freely with those adjacent). Part of its wall, near its attendant artery, is thickened. (×22)

the space. However, in some cases numerous isolated areas of epithelium lined masses of connective tissue in the foci; from these areas cords of epithelium occasionally extended into the fibrous scar tissue, ending blindly (Figure IX).

The bronchioles themselves showed much evidence of old inflammatory damage with thickened walls and increased collagenous tissue. The two divisions of bronchioles just proximal to the communications usually contained little muscle, and in some instances only occasional muscle cells were found (Figure X). The elastic tissue of the wall was usually partially absent; but where the original subepithelial net could be recognized, it was possible, in many examples, to discover evidence of partial obliteration of the original lumen.

The epithelium lining of the bronchioles was often irregular, being flattened and tenuous over some areas of the wall (Figure X). At other sites diverticula were seen, ending in solid cords of epithelial cells, some of which were continuous with epithelium lining small areas of adjacent air spaces.

Larger bronchioles proximal to those within the focus showed less change, but were also damaged to a variable extent; some of these bronchioles were thin-walled and irregularly dilated, while others had thickened walls containing numerous diverticula. In more advanced disease, even small bronchi occassionally showed evidence of damage.

Many of these diverticula were regarded as secondary formations, but reconstruction of serial sections showed that a few were the proximal part of obliterated bronchioles, the diverticulum continuing as a cord of scar tissue which became related to arterial branches (Figure XI). This scar tissue ended in the walls of dilated spaces, which were often lined by flattened or cuboidal epithelium.

Definite identification of obliterated bronchioles in tissue showing macroscopic focal emphysema was limited to those which were originally of large size—often first or second order bronchioles (Figure XI). Where they were found, the lung distally was so distorted that interlobular septa could not be recognized, and the spaces to which they led often communicated with other lobules.

Search was made for smaller bronchioles that had been obliterated. Many structures were seen which may have been obliterated

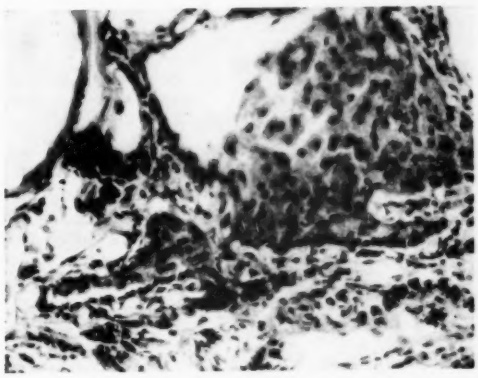


FIGURE XIII

Photomicrograph showing the area of thickened wall in Figure XII with higher magnification. An epithelium-lined recess communicates with a larger epithelium-lined space containing numerous macrophages. (At many sites it was not possible to distinguish epithelium from macrophages.) The surrounding connective tissue contains black pigment masses. Little muscle was seen. (×120)

bronchioles; one example showed a mass of epithelial cells extending peripherally from a recess deep in the outer wall of a patent bronchiole into an adjacent air space (Figures XII and XIII); the origin of this bizarre

formation, even after detailed reconstruction of the surrounding passages, was not indubitable, since no structure resembling normal peripheral passages could be identified. At the same time, the appearances were best explained on the basis of this being an obliterated passage. Thus, even in examples where a bronchiole arising from a terminal bronchus was found to

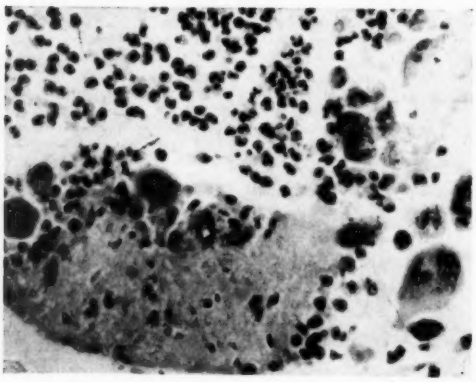


FIGURE XIV

Photomicrograph showing inflammatory exudate in an emphysematous air space. An almost structureless mass of red cells (below) is surrounded by macrophages, many of which are in giant-cell form. Most macrophages contain dense granular material, and their cytoplasm contained much ferric iron. (×120)

have no more than three divisions before entering peripheral spaces, the circumstantial evidence for bronchiolar obliteration was considerable though the numerous bronchioles that must have been obliterated in the past could not be positively identified at this stage of the disease.

BLACK PIGMENT IN THE LUNGS

Distribution

All adult lungs examined showed some black pigmentation macroscopically, whether they exhibited focal emphysema or not. Most pigment was seen on the periphery of secondary lobules (particularly near veins and subpleurally) and in the lymph nodes within the lung, at the hila and in the mediastinum. Variable but generally smaller amounts were also visible, related to the bronchovascular radicles in the middle of the lobules (McLean, 1956*a*).

Histologically, most of the pigment was seen as discrete, densely black, somewhat irregular masses averaging 15μ in diameter. That in the middle of the lobule was found largely in the connective tissue of the outer walls of bronchioles and arteries; in "normal" lungs little was obvious proximal to the preterminal bronchioles or distal to the second order of respiratory bronchioles.

In sections of material exhibiting focal lesions, the site and amount of pigment varied greatly. As has been indicated, gross focal change was sometimes not associated with notably excessive depositions of pigment in the focus, although, generally, the more circumscribed the dilatation of air spaces in the lobules the more pigment was present. In many examples of focal change the walls of the dilated air spaces contained much pigment, sometimes in dense masses and sometimes scattered through collagenous scar tissue; in others, even though collagenous bands were present, little was found.

The Nature of the Pigment

In certain circumstances there could be no doubt that such black material was exogenous. The lungs of the two coal-miners showed such large quantities of pigment in the walls of the dilated spaces (Figure VIII) that the source of

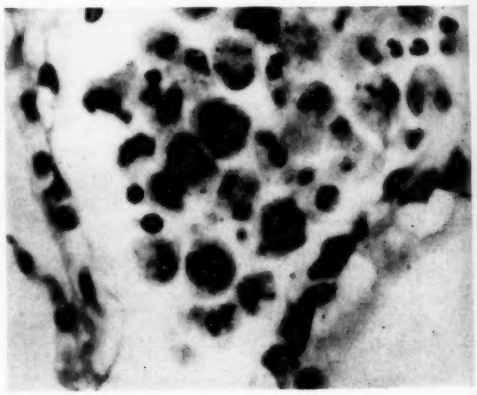


FIGURE XV

Photomicrograph showing inflammatory exudate in an alveolus adjacent to a respiratory bronchiole occluded with exudate. Numerous large macrophages diffusely stained with iron pigment and containing tiny black granules are present, as well as occasional polymorphonuclear cells. (×210)

the pigment must necessarily have been inhaled coal dust. In other circumstances, however, an endogenous source was indicated.

In many examples of acute bronchiolitis, where the inflammatory process involved the passages surrounding the terminal bronchiole, erythrocytes were abundant in the exudate.

Among the polymorphonuclear leucocytes, macrophages with dark red-brown cytoplasm were frequently seen with hæmatoxylin staining (Figures XIV and XV) and an intense blue colour with the Prussian blue reaction. Such macrophages were particularly abundant, often showing giant-cell formation, where they were aggregated about a disorganized mass of red cells (Figure XIV). This material in the cytoplasm was regarded as being hæmosiderin.

Most of these macrophages also contained dark brown to black granules, which, when present in small numbers, could be seen to vary in size from about 0·5μ (the limit of resolution) to 2μ, the larger particles often appearing to be agglomerates of the smaller (Figure XV). In the same section many of these dark cells were packed with granules; extracellular granules were not seen.

In the material examined, wherever a group of macrophages was found showing this diffuse brown colour of the cytoplasm (which always gave a positive iron reaction), many or all of the cells contained fine brown to black granules.

In older lesions, where exudate and polymorphonuclear leucocytes were no longer apparent, similar macrophages were found

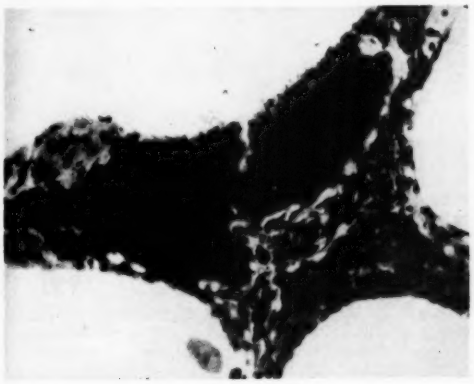


FIGURE XVI

Photomicrograph showing pigmented tissue towards the middle of an area of focal emphysema. The wall clearly consists of an older part together with two more recent masses of cells, packed with black pigment, occluding communications and filling recesses. (×120)

lying against the walls of air spaces either singly or in masses. In normal lungs these cells were found mostly in the alveoli of respiratory bronchioles or their immediate divisions, but sometimes were more peripheral, even lying in air spaces in the border of the lobule.

In lungs showing early focal change a similar distribution was obvious, and groups of pigment-filled macrophages giving a strongly positive Prussian blue reaction were commonly found. Such masses, when seen on the walls of dilated air spaces, often filled and rounded off recesses and, also, occluded small passages (Figure XVI).

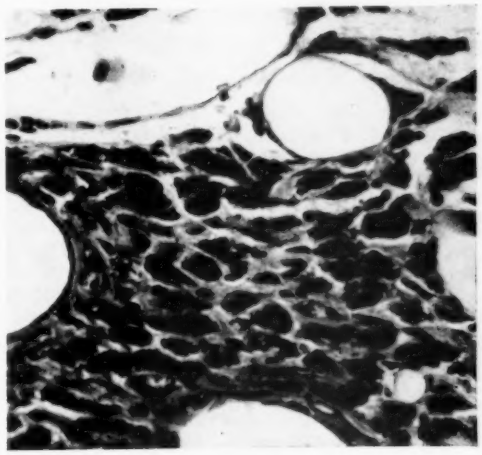


FIGURE XVII

Photomicrograph showing a dense mass of pigmented tissue in an area of focal emphysema. The pigment clearly consists of fine granules and is intracellular, obscuring cell detail. The intercellular substance is collagenous, and there are several thin-walled capillaries in the tissue. (×200)

Similar masses were found applied to the outer walls of bronchioles. On occasions the macrophages on the surface appeared to form an epithelial layer (Figure XVI). The amount of pigment in these macrophages varied considerably, some being so packed that the cell outline was scarcely visible (Figure XVI). Even in examples where the macrophages were packed with fine granules, the result of the test for ferric iron remained positive; but where the cell was completely black and opaque, the blue coloration could no longer be seen.

Although, exceptionally, pigment-filled macrophages were seen in lymphatic vessels, it was obvious that most of the cells did not enter the interstitial tissue to reach the lymphatics, but rather remained in the recesses outside the original tissues, becoming progressively denser. At several sites masses of these cells were seen with a ground substance, sometimes containing collagen, between them. Small capillaries were sometimes visible at this stage (Figure XVII). In some examples this appearance was probably

due to the collapsed walls of spaces originally containing these macrophages, but in others there seemed little doubt that all this tissue was a new formation.

These observations were studied concurrently with the fate of mucus-laden phagocytes that were also seen frequently in the passages immediately beyond the terminal bronchiole. Masses of such cells were sometimes observed in " normal " lungs, filling the alveoli of respiratory bronchioles and their immediate branches and, in areas of focal emphysema, filling mural defects and small passages. In " normal " lung, single macrophages were seen lying against alveolar walls (but not in the interstitial tissue), most of these cells being somewhat flattened and often indistinguishable from septal cells except for their content of granular material giving a positive periodic acid-Schiff reaction. However, such cells were seen only where there was other evidence of recent mucus aspiration in that lobule; so it was concluded that the polysaccharide moiety of mucus is relatively rapidly attacked by the enzymes of macrophages.

Thus, after acute bronchiolitis in which the inflammatory process had spread beyond the terminal bronchioles, slow or incomplete resolution, with filling of recesses and even occlusion of passages, was common, the new tissue often being " labelled " by black granules. In focal emphysema this process was often much accentuated, even with occlusion of the communications of bronchioles with the " pool " (Figure VI).

Discussion

The essential change in acute focal emphysema is dilatation of the air passages immediately distal to an obstruction, the dilatation being most obvious where the collateral air supply to the affected area has had to traverse a tortuous passage involving many small passages and, through alveolar pores, many alveolar walls.

In a previous presentation of homœostatic mechanisms in the air passages, it was emphasized that air pressure distal to such an obstruction was raised in quiet respiration, and also that this pressure rose greatly with coughing. This phenomenon was explained by air-trapping, air entering the area through dilated passages and open alveolar pores with inspiration, the resistance to outflow rising with expiration due to reduction in the lumens of the passages of exit and closure of the pores through which the air must pass (Figure XVIII). Coughing obviously greatly accentuates this " air-trapping ". Observations supporting this were made by Macklin (1936) and Loosli (1937), who showed, in histological preparations, that alveolar pores increased in size and were more easily seen in well inflated lungs than in less inflated specimens.

In these circumstances the maximum effect of " air-trapping " would be felt in those passages furthest removed from surrounding normally ventilated lung units (Figure XVIII). Where the distribution of a lobular bronchus was occluded (as was usually the case), those passages towards the centre of the lobule just beyond the obstructed divisions obviously would be the most affected.

Free collateral ventilation would be further inhibited and greater " air-trapping " result if the pores in the alveoli lining these passages were occluded (for instance, by exudate or macrophages), so that aeration occurred only by its peripheral branches and not also through its walls.

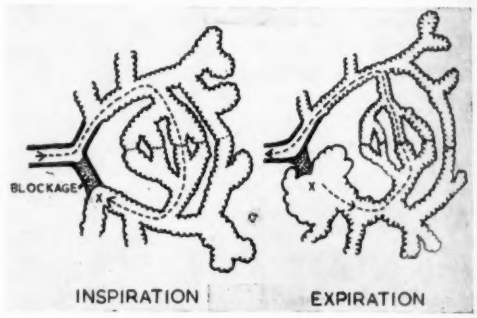


Figure XVIII

Highly diagrammatic representation of " trapping " of collaterally ventilated air. A plug occludes a bronchiole, more distal passages being aerated by collateral ventilation through alveolar pores from an adjacent group of passages. With inspiration the air enters freely through dilated passages and widely open pores; with expiration air is readily expelled from the normally ventilated area, but as the passages and pores become reduced in size, air is trapped, particularly in the area farthest removed from the source of collateral ventilation (X). The more tortuous the path, the greater the air-trapping; if the path is very tortuous, air may enter the area only during expiration

An objection could be raised that this dilatation observed was artefact, but, since the phenomenon was found in tissue which was fixed by simple immersion in fixative, by endobronchial injection of fixative into excised lungs or by intravenous injection of fixative into the cadaver, it does not require serious attention.

The main features of early lesions of chronic focal emphysema were two: the formation of the "common pool" and old inflammatory damage of bronchioles leading to the "pool".

Breakdown of alveolar walls was obvious in the earliest lesions and was seen in those alveoli which are, in essence, thin-walled diverticula or air passages; similar observations were made by Cugudda (1949). This disruption affected mainly the respiratory bronchioles, so that they communicated freely with adjacent passages, often with only a few musculo-elastic strands remaining. This proceeded in more advanced lesions until a large space (designated "common pool") was formed, first around terminal bronchioles and then in the whole inner zone of the secondary lobule where the terminal bronchioles ended.

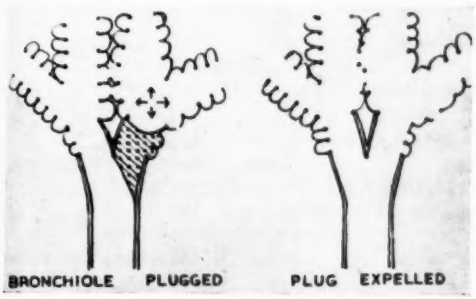


FIGURE XIX

A much simplified diagram showing "air-trapping" in the passages immediately distal to a plug in a bronchiole. Some dilatation of the passages and disruption of the alveoli in their walls has occurred (left). With expulsion of the plug some permanent change may remain (right)

In early cases the communications of the "pool" with more peripheral passages were completely free, and only in the most advanced focal change were these communications reduced in their lumens or in any way tortuous.

The essential effect of this breakdown of alveolar walls (which often extended further into the lobule in more advanced forms of emphysema) is to produce the equivalent of enlargement of the alveolar pores. Relatively large defects in alveolar walls in emphysematous lungs were noted by Waters (1862), Sudsuki (1899), Orsós (1907) and Macklin (1936), but none of these authors indicated that not only are alveolar walls disrupted but also the musculo-elastic mesh forming the wall of the passage from which the alveoli arise is similarly affected.

It is generally supposed that the stimulus inducing this disruption is excessive pressure inside the passage (Macklin, 1936; Loosli, 1937). Personal observations include none which argues against this proposition. The main problem in early focal emphysema is to decide what forces could be operative to produce such a localized change, since there is no airway obstruction evident proximally or distally.

Wherever dilated and disrupted passages were found, bronchiolar damage was apparent proximally, but often was not seen in adjacent normal lobules, and in all early cases of focal emphysema the bronchioles leading to emphysematous spaces were abnormal.

Such old damage clearly resulted from episodes of inflammation. Acute bronchiolitis has been shown to be essentially an obstructive lesion with completely occluding plugs extending distally at least as far as first order bronchioles (McLean, 1956*b*); as most episodes of acute bronchiolitis probably resolve completely, residual bronchiolar damage can be regarded as the result of prolonged, repeated or severe damaging inflammation and, most probably, of all three (McLean, 1957).

Thus the basis of an hypothesis of pathogenesis emerges. If the above explanation for acute focal emphysema be accepted, it can be applied directly to the chronic form. Excessive intraluminal pressure in the passages beyond obstructions in acute bronchiolitis may well produce rupture of the alveolar walls in this acute stage, and, particularly if some exudate is present in these passages, the distorted and disrupted passages may not resume their normal appearance after the plug is expelled and inflammation subsides (Figure XIX). The forces acting on the walls would clearly be greater with coughing—an act which ordinarily expels the plug but sometimes does not. Prolonged or repeated obstruction thus would not only result in greater permanent damage to the smallest bronchioles but also increase the total duration of stress on alveolar walls. Disruption of the walls would necessarily be related to the force applied and its total duration of action.

Temporary bronchiolar obstruction is, however, clearly only the basis of an hypothesis. It is inadequate to explain, for instance, absence of significant macroscopic emphysema in many cases of prolonged asthma in which extensive mucus obstruction of small bronchioles undoubtedly occurs very much more frequently than acute bronchiolitis occurs in non-asthmatic patients with macroscopic focal emphysema. Similarly, it does not explain many of the

pathological features of the disease that have been presented here.

The relation of bronchiolar obliteration to emphysema is less obvious, but careful observation demonstrates its significance.

Partial organic obliteration of bronchioles, producing a "ball-valve" effect with over-inflation of the lung distally, was proposed by Spain and Kaufman (1953) to be the basic lesion of emphysema. Study of early lesions refutes this concept, since even slight partial obliteration, which was readily recognizable, was often not present in bronchioles in affected secondary lobules. Also, partial obliteration sufficient to occlude the lumen significantly was not seen in early focal lesions and was by no means common even in macroscopic lesions.

Complete bronchiolar obliteration presents a more difficult problem, since, if temporary obstruction can result in stresses being exerted on the walls of passages distal to the obstruction, it is clear that the effects of such stresses would be much greater if the obstruction became permanent.

Lesions identified as obliterated bronchioles were found in most emphysematous lungs. In the earliest focal changes bronchioles larger that preterminal size were rarely involved, but in more advanced disease even the largest bronchioles were found obliterated. Thus it can be stated that with the progress of emphysema the level of bronchiolar damage and obliteration ascends the bronchial tree.

Whether the extent of bronchiolar obliteration proceeds *pari passu* with development of emphysema could not be directly demonstrated, but is suggested by progressive reduction of bronchiolar division. However, in a previous study of the evolution of bronchiolar obliteration (McLean, 1957) it was concluded, despite the observation that most old lesions were microscopically inconspicuous or even imperceptible, that the maximal incidence of obliteration occurred in the smallest bronchioles (in particular, the first and second order respiratory bronchioles) with progressively fewer lesions in the larger passages. This distribution of bronchiolar obliteration applied whether the lung distally was aerated or not and was independent of the severity of obliterative changes.

This evidence indicates that permanent obliteration of bronchioles is an integral part of the changes associated with emphysema. In so far as obliteration develops as a sequel of acute bronchiolitis only where the occluded lumen is not cleared, and since emphysema does not develop if the lumen is rapidly cleared, bronchiolar obliteration must be regarded as a primary phenomenon in the causation of focal emphysema.

Whether such permanent obliteration is either inevitably or extensively present in the early stages of emphysema could not be definitely stated, but, since in these early lesions the smallest old obliteration recognized was that of a preterminal bronchiole and its branches, it may reasonably be assumed that many more shorter arborizations (for instance, a first order respiratory bronchiole and its immediate divisions) had been obliterated but were imperceptible. Thus it seems probable that permanent obliteration is significant even in the earliest stages of chronic focal emphysema (Figure XVIII). Further indirect evidence is afforded by the observation that patent bronchioles in early lesions show evidence of old inflammatory damage. Such damage is an indication of past bronchiolitis that was prolonged, repeated or severe—the very circumstances in which complete obliteration was found most commonly (McLean, 1957).

The manner in which these obliterated shorter arborizations could become imperceptible is best illustrated diagrammatically. Dilatation of the spaces distally and rupture of thin walls may result in the development of secondary communications which can easily be mistaken for original passages (Figure XIX); this illusion is heightened if part of the communication becomes epithelialized. Indeed, even when a longer arborization of an obliterated bronchiole was definitely recognized, the spaces to which the terminations of the scar ultimately became related were often continuous with those supplied by patent bronchioles; so that, in reconstructions, the peripheral passages of the patent bronchioles communicated by distorted passages, which led to all the spaces surrounding the terminations of the scar of the obliterated bronchiole. Thus when a bronchiole becomes completely obstructed, its peripheral path may remain patent and be supplied, albeit by pathways of abnormal structure, by neighbouring still patent bronchioles.

Therefore the hypothesis, that early chronic focal emphysema is due to complete bronchiolar obstruction of either temporary or permanent nature, is consistent with the observations recorded.

Most lungs showing focal emphysema are relatively uniformly affected (McLean, 1956*a*). This would be expected, since collateral ventilation would be relatively free (and therefore "air-trapping" minimal) in the air passages

beyond an isolated obstructive lesion in a small bronchiole. The common form of acute bronchiolitis being generally an extensive obstructive lesion, many lobules are simultaneously obstructed; and if obstruction is prolonged, repeated or severe, permanent

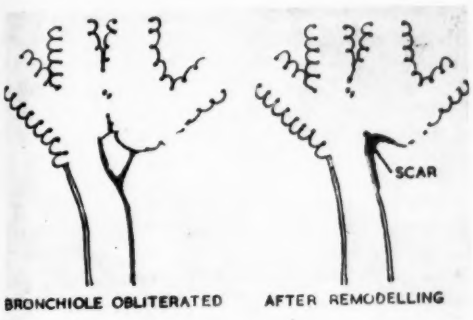


FIGURE XX

Diagram showing inflammatory obliteration of the occluded bronchiole in Figure XIX. After remodelling, the scar is inconspicuous. "Air-trapping" persists until sufficient breakdown of alveolar walls permits free collateral ventilation. The musculo-elastic mesh remains as bare strands crossing a "common pool"

obliteration of small bronchioles will be widely disseminated. For these reasons, since collateral ventilation between lobules is unrestricted in focal emphysema, significant "air-trapping" depends essentially on widespread bronchiolar lesions affecting most of the lobules in the part of the lung concerned. Thus the extent, and therefore the effects, of "air-trapping" would be relatively uniform between lobules.

The variation in the forms of focal emphysema requires explanation. In one group the centrolobular lesions become more diffuse with expansion of the "common pool". This group has been described, in the macroscopic descriptions of the variants of the disease, as the early form of "fine strand" emphysema (McLean, 1956*a*). In these lobules there was little fibrosis or pigmentation in the residual strands in the pool; so it may be assumed that, in the preceding incidents of acute bronchiolitis which resulted in the bronchiolar damage, there was either little exudate (or other material) peripheral to the respiratory bronchioles, or that most such material was subsequently resorbed or expelled.

Passages distal to obstructed bronchioles would be subject to distending stresses, with the result that many of the thin-walled alveoli forming their walls would be disrupted. This would allow freer collateral ventilation on all sides of the passage, the lumen being represented near the obstruction by irregular, bare musculo-elastic bands, seemingly crossing the middle of an emphysematous space (Figure XX). Extension of the process would then necessitate further obstructive phenomena in that and adjacent lobules.

The other group was characteristically represented by well-developed macroscopic focal emphysema, in which the dilated spaces were sharply localized to the middle of the lobule. In these lesions the residual tissue in the foci was relatively coarse and often fibrous with, in many cases, masses of black pigment intermingled. This excess tissue presumably arose as a sequel to acute bronchiolitis in which there was incomplete resorption of material in the passages beyond the complete obstruction. Part of such tissue could be formed by organization of inflammatory exudate and part from accumulation of macrophages containing either hæmosiderin granules derived from red cells in the exudate or coincidently present exogenous material such as soot or coal dust. All of these fill recesses and bridge spaces in the affected

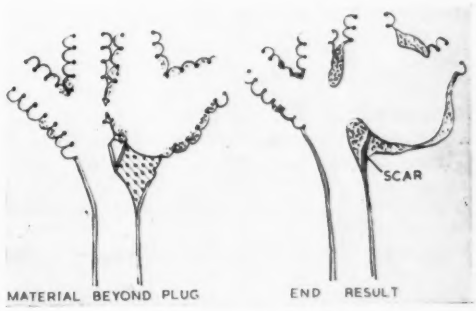


FIGURE XXI

Diagram similar to those in Figures XIX and XX except that here non-resorbable material, such as exogenous dusts or blood pigment, is present within the passages distal to the obstruction during the acute inflammatory phase (left). This material occludes alveolar pores and therefore reduces collateral ventilation; air-trapping and therefore dilatation are increased. At a later stage (right), residual structures are unrecognizable; if the bronchiole is obliterated, its remnants are mingled with pigment-containing scar tissue. Note that, particularly if some epithelialization occurs, there may appear to be no reduction in passages, and secondary communications may be termed respiratory bronchioles

passages and so reduce collateral ventilation in the area. This would result in accentuation of air trapping and, because of the thickening of walls of the passages, lessen the tendency for disruption (Figure XXI). Thus relatively

localized air-trapping results, and with repeated incidents grossly dilated spaces can form.

In this way large dilated spaces would be apparent at a stage where, with a similar extent of proximal obstruction (and bronchiolar damage), the more diffuse form would show less change because of the freer collateral ventilation beyond obstructed passages. Thus, where deposition of material in the walls of the dilated spaces is excessive, as occurs in coal-miners, only moderate inflammatory damage to bronchioles may be associated with macroscopically visible emphysema.

Observations made here suggest that part of the black pigmentation in lungs is endogenous and is due to hæmosiderin as well as to exogenous dusts (particularly those which are essentially carbon, such as soot, smoke and coal-dust).

This proposition is by no means new, but has scarcely been considered outside the German literature. Virchow (1847), amongst others, originally thought that all the "*Lungenschwarz*" was altered blood. The observations on which he based his opinions were reinforced by others such as Rebsamen (1862) and Schmidt (1889, 1901), who observed similar transitions from obvious brown granular hæmosiderin to condensed black masses similar to those described here. Subsequently Virchow (1866) admitted that exogenous carbon contributed to pulmonary pigmentation to a variable extent and was sometimes obvious microscopically as relatively large angular particles in the lungs of coal-miners. However, he stated firmly that he was not satisfied that all fine granules of pigment were necessarily carbon.

The use of the Prussian-blue reaction for ferric iron, introduced by Perls (1867), merely confirmed that the brown pigment contained ferric iron and therefore was probably hæmosiderin. The nature of the black granules could not be revealed by this method. However, Scheid (1932) demonstrated by microincineration that, in sections of lungs containing the relatively small amounts of black pigment found in the lungs of the average adult, the ash of the granules contained large amounts of iron, relatively much more than was present in cells, the cytoplasm of which was diffusely stained with hæmosiderin. Therefore it must be concluded that these black granules are rich in an iron-containing material of endogenous origin.

The crucial point is whether any black granules are composed entirely of hæmosiderin. Scheid found that treatment with 20% hydrochloric acid solution or *aqua regia* vapour removed the iron (as demonstrated by absence of ash after microincineration), but the granules remained black. He therefore concluded that all such granules contained carbon and, following Neumann's (1900) hypothesis, held that the carbon particles adsorbed the hæmosiderin. This conclusion is, however, fallacious, since Lillie (1939) has shown that aggregates of indubitable hæmosiderin in organs other than lung did not alter in colour after removal of all demonstrable iron by acid. He designated the substance remaining "aposiderin" and considered it to be a protein complex, which he showed to be resistant to prolonged treatment with acids and alkalis and also many oxidizing and reducing agents.

Therefore only limited conclusions are possible regarding the nature of the finely granular aggregate of black pigment found so commonly in lung. Some part is definitely endogenous, certainly a form of hæmosiderin, although many such aggregates may contain carbon. On the other hand, there can be little doubt that the large amounts of black pigment in the lungs of coal-miners are at least largely carbon in the form of coal-dust.

The origin of the hæmosiderin would appear to be clear. Although Virchow (1847) and most subsequent authors stressed its formation in congested lung, in which the hæmosiderin-containing "heart failure" cells are obviously macrophages containing products of ingested red cells, little emphasis has been placed on acute bronchiolitis in which, when inflammation extends beyond the terminal bronchiole, red cells are often seen amongst the exudate.

After subsidence of the acute inflammation, the pigment-containing macrophages were found largely in the walls of the passages (including the outer walls of bronchioles), only a fraction of them entering the lymphatic vessels. The significant point is that most of the cells on the walls remain static. Where they occur in masses, the macrophages may assume the function of fibroblasts, possibly because of the relatively acidic environment due to their remoteness from lung capillaries—a form of metaplasia which has been discussed previously (McLean, 1957). In any case, the regular demonstration of iron in most such masses by Scheid (1932) indicates that the pigment is static and is removed from these deposits at an insignificant rate, if at all. This suggests that the process of darkening of the pigment (until it is black) may be a function of the duration of deposition in areas which, until they are subsequently vascularized, are in effect outside the body.

Thus the hæmosiderin component of black pigments is intimately related to the inflam-

matory basis on which the theory of pathogenesis rests.

Inhaled carbon is less directly related to acute bronchiolitis. Scott *et alii* (1949) have shown conclusively that inhaled particulate matter was deposited maximally in the larger air passages with only a small amount reaching the terminal passages; dust proximal to the terminal bronchiole was rapidly expelled, but even after months much of the fraction that entered the more peripheral passages still remained. Heppleston (1953, 1954*a*) observed that the initial deposition was relatively uniform in the distal passages but after some time was largely found, in normal lungs, filling the alveoli of the respiratory bronchioles. This could be explained by the observation of Macklin (1955) that the layer of fluid on the terminal air passages moves constantly centripetally to the region of the respiratory bronchioles, where, he states, it enters " sumps " or defects in the walls and from thence gains access to the lymphatic vessels.

Opinion is divided whether dust is phagocytosed in the alveoli before or after it enters the interstitial tissue, but most writers hold that intraalveolar phagocytosis occurs in man (von Hayek, 1952; Heppleston, 1954*a*). A point not mentioned by these authors in the discussion of phagocytosis of inhaled dusts is that mucus can be aspirated into the terminal air passages, where it is rapidly phagocytosed (McLean, 1956*b*) and would contain much dust if the subject were exposed at the time to dusty atmospheres. Such a phenomenon would account for the clearly localized inhaled masses of pigment illustrated by Heppleston in man (Heppleston, 1953, Plate 69, Figure IV) and in the experimental animal (Heppleston, 1954*b*, Plate 76, Figures II and III).

The dust that does not enter the lymphatics and is retained in the area becomes eventually incorporated in connective tissue containing a variable amount of collagen. As muscle and elastic fibres are occasionally found in these structures (Heppleston, 1954*a*), they are undoubtedly formed partly by the condensation and organization of the walls of original structures. Thus focal emphysema in coal-miners does not differ essentially from the commoner form found in many city-dwelling adults except for the greater mass of pigment in the foci which are therefore rendered more obvious. As coal-miners are no less liable to recurrent attacks of acute bronchiolitis manifested as " febrile catarrh ", colds, influenza and " acute bronchitis " than are the rest of the population, there appears to be no necessity to invoke a separate theory of pathogenesis for this variation of focal emphysema.

Thus a theory of pathogenesis has been evolved which is consistent with observations on many aspects of the condition. In essence it rests on two basic concepts: first, that forces exist which act to increase the pressure in passages peripheral to sites of obstruction in bronchioles and, second, that diffuse permanent obliteration of the smallest bronchioles is widespread in emphysema but is not easily or directly demonstrable.

Previous hypotheses of the origin of emphysema are, it is generally agreed, unsatisfactory (Christie, 1954); and, since morphologists have been seeking a satisfactory hypothesis for over a hundred years, it should be clear that the basic phenomena underlying the process are by no means obvious and probably not apparent in well-developed lesions. The concept of diffuse obliterative changes in bronchioles is concerned with processes which, judging by their neglect, are not obvious and which are not apparent in the fully developed disease.

Few previous hypotheses are in any way consistent with the observations on the early stages of the disease. Those based on " ball-valve " partial bronchiolar obstruction in general and partial bronchiolar obliteration in particular (Spain and Kaufman, 1953) have already been shown not to conform with observations. " Chronic bronchitis " was associated neither clinically nor morphologically with the earliest lesions and so cannot be regarded as preceding the earliest structural changes (although it may precede the development of clinical emphysema). Its association with emphysema therefore cannot be primary.

Hypotheses based on observations of the earliest lesions of emphysema are few. Heppleston (1947, 1951, 1953, 1954*a*) carefully studied the three-dimensional histology of focal emphysema in coal-workers. He concluded that the focal lesion in these cases was due simply to the mechanical effect of the relatively massive deposits of coal-dust in the walls of respiratory bronchioles.

As such a conclusion differs so greatly from that reached here, it was necessary to examine this problem closely. The most serious deficiency in the basic approach was that those cases exhibiting focal emphysema in which insignificant amounts of pigment were found (Gough, 1952) were ignored—such lesions obviously are not caused by the mechanical effects of coal dust. Also, a form of the condition in which large deposits of black

pigment obscure what little remains of original structures is not ideal material for study of minute changes, especially in such a tissue as the lung.

No true intermediate forms between normal and emphysematous lung were described or illustrated by Heppleston; this must be the reason why the author felt justified in the incorrect assumption that the communications of the bronchioles with the dilated spaces were the original respiratory bronchioles arising from the original terminal bronchioles, even though they bore no demonstrable resemblance to the original structures but were simply spaces with which altered bronchioles communicated. Basing his observations on this assumption, he then concluded that there was no evidence of obliteration of bronchioles.

Heppleston (1947) showed that these focal lesions in coal-workers contained insignificant amounts of silica and that the quantity of silica found in a focus bore no relation to the extent of the lesion. Since it was also concluded that infection played no part in the pathogenesis of the lesion (Heppleston, 1954*a*), no explanation is therefore offered for the fibrosis and the dissolution of specialized tissues that are observed both in the walls of the dilated spaces and in the passages leading to them. Thus, in view of these deficiencies, his hypothesis is unacceptable.

Acknowledgements

The work embodied in this paper was carried out while the author was in receipt of a grant from the National Health and Medical Research Council.

References

Christie, R. V. (1954), "Emphysema of the Lungs", in "Non-Tuberculous Diseases of the Lungs", edited by Banyai, A. L. Charles C. Thomas, Springfield, Illinois, U.S.A.

Cugudda, E. (1949), "*La forma dei lumi bronchiolo-alveolari nell'enfisema essenziale*", *Riv. Anat. patol.* **1**, 252; abstract in *Excerpta med. (Amst.), Sect. V* (1950), **3**, 614.

Gömöri, G. (1936), "Microtechnical Demonstration of Iron", *Am. J. Path.*, **12**, 655.

Gough, J. (1952), "Discussion of the Diagnosis of Emphysema: The Pathological Diagnosis of Emphysema", *Proc. Roy. Soc. Med.*, **45**, 576.

Heppleston, A. G. (1947), "The Essential Lesion of Pneumokoniosis in Welsh Coal Workers", *J. Path. & Bact.*, **59**, 453.

Heppleston, A. G. (1951), "Coal Workers' Pneumokoniosis", *Arch. Indust. Hyg.*, **4**, 270.

Heppleston, A. G. (1953), "The Pathological Anatomy of Simple Pneumokoniosis in Coal Workers", *J. Path. & Bact.*, **66**, 235.

Heppleston, A. G. (1954*a*), "The Pathogenesis of Simple Pneumokoniosis in Coal Workers", *J. Path. & Bact.*, **67**, 51.

Heppleston, A. G. (1954*b*), "Changes in the Lungs of Rabbits and Ponies Inhaling Dust Underground", *J. Path. & Bact.*, **67**, 349.

Lillie, R. D. (1939), "Experiments on the Solubility of Hemosiderin in Acids and Other Reagents during and after Various Fixations", *Am. J. Path.*, **15**, 225.

Loosli, C. G. (1937), "Interalveolar Communications in Normal and in Pathologic Mammalian Lungs", *Arch. Path.*, **24**, 743.

Macklin, C. C. (1936), "Alveolar Pores and Their Significance in the Human Lung", *Arch. Path.*, **21**, 202.

Macklin, C. C. (1955), "Pulmonary Sumps, Dust Accumulations, Alveolar Fluid and Lymph Vessels", *Acta anat., Basel*, **23**, 1.

McLean, K. H. (1956*a*), "The Macroscopic Anatomy of Pulmonary Emphysema", *Australasian Ann. Med.*, **5**, 73.

McLean, K. H. (1956*b*), "The Pathology of Acute Bronchiolitis—A Study of its Evolution. Part I: The Exudative Phase", *Australasian Ann. Med.*, **5**, 254.

McLean, K. H. (1957), "Pathology of Acute Bronchiolitis—A Study of Its Evolution. Part II: The Repair Phase", *Australasian Ann. Med.*, **6**, 29.

Neumann, E. (1900), "*Das Pigment der braunen Lungen-induration*", *Virchows Arch.*, **161**, 422.

Orsós, F. (1907), "*Ueber das elastische Gerüst der normalen und der emphysematösen Lunge*", *Beitr. Path. Anat.*, **41**, 95.

Perls, M. (1867), "*Nachweis von Eisenoxyd in gewissen Pigmenten*", *Virchows Arch.*, **39**, 42.

Rebsamen, C. A. (1862), "*Die Melanose der menschlichen Bronchialdrüsen*", *Virchows Arch.*, **24**, 92.

Scheid, K. F. (1932), "*Über exogene und endogene Eisenbelagerungen in der Lunge*", *Beitr. path. Anat.*, **88**, 224.

Schmidt, M. B. (1889), "*Ueber die Verwantschaft der hämatogenen und autochthonen Pigmente und deren Stellung zum sogenannten Hämosiderin*", *Virchows Arch.*, **115**, 397.

Schmidt, M. B. (1901), "*Ueber Hämosiderin und Melanin*", *Virchows Arch.*, **163**, 151.

Scott, K. G., Axelrod, Dorothy, Crowley, Josephine and Hamilton, J. G. (1949), "Deposition and Fate of Plutonium, Uranium and Their Fission Products Inhaled as Aerosols by Rats and Man", *Arch. Path.*, **48**, 31.

Spain, D. M., and Kaufman, G. (1953), "The Basic Lesion in Pulmonary Emphysema", *Am. Rev. Tuberc.*, **68**, 24.

Sudsuki, K. (1899), "*Ueber Lungen-emphysem*", *Virchows Arch.*, **157**, 438.

Virchow, R. (1847), "*Die pathologischen Pigmente*", *Virchows Arch.*, **1**, 379.

Virchow, R. (1866), "*Ueber das Lungenschwarz*", *Virchows Arch.*, **35**, 186.

von Hayek, H. (1952), "*Die menschliche Lunge und ihre Gefässe, ihr Bau unter besonderer Berücksichtigung der Funktion*", *Ergebn. Anat. Entwgesch.*, **34**, 144.

Waters, A. T. H. (1862), "Emphysema of the Lungs", Churchill, London.

TOXOPLASMOSIS : THE DIAGNOSIS OF CLINICAL AND LATENT INFECTION IN AUSTRALIA[1]

ALISON GARVEN[2]
From the Footscray and District Hospital, Melbourne

SUMMARY

A summary of the clinical features of toxoplasmosis is given and the use of diagnostic tests is described. The disease presents most commonly as a congenital infection, which is transmitted to a fœtus from a mother who is suffering from an acute asymptomatic infection. The infants suffer most commonly from hydrocephalus, convulsions, chorio-retinitis and cerebral calcification. In the acquired form it may present as a typhus-like illness with fever, maculo-papular rash and symptoms of encephalitis and pneumonia. An antibody survey of a sample of the Australian population is presented and the nature of the dye test outlined. It was found that 35% of the adult population had cytoplasm-modifying antibodies in their serum. The interpretation of these findings is discussed.

TOXOPLASMOSIS is a disease which has been recognized and studied in most countries for many years, but for some reason it has aroused interest in Australia only during the last ten years. It is caused by a protozoan called *Toxoplasma gondii*, a crescent-shaped organism, about 5μ in length, which is an obligate intracellular parasite. It has no species specificity, and all mammals are susceptible, although the pigeon is apparently the only bird that is affected. Nor has it a tissue specificity, but the organs most severely affected differ from one species to another. It multiplies by binary fission within both the parenchymal and reticulo-endothelial cells until nothing is left of the cell but a thin membrane surrounding a colony of toxoplasmas. This is called a pseudocyst, and it apparently resists both the inward passage of antibodies and chemotherapeutic agents and the outward passage of parasite products, so that there is no surrounding tissue reaction. When the infection is in the acute stage, the organisms lie free in the tissues as well as inside the cells, and it is at this stage only that they are susceptible to chemotherapy. In the chronic stage the organisms are found only in the cystic form, which can be demonstrated in histological preparations either by a silver stain or by a PAS stain.

CLINICAL FEATURES

Clinically the disease presents in a variety of forms. The most commonly recognized form is the congenital infection, which is transmitted to a fœtus from a mother who is suffering from an acute asymptomatic infection. It is unknown for a mother to have more than one child suffering from the congenital form of the disease, so that a good prognosis for future children can safely be given. The infants suffer from hydrocephalus or microcephaly, microphthalmos and other ocular defects, and almost invariably chorio-retinitis involving the macula, although this may not develop until after birth. Cerebral calcification is seen in about 60% of cases and appears as linear streaks in the cortex and basal ganglia. Convulsions are frequent, and in later childhood there is usually mental retardation and spasticity as well. Persistent jaundice and enlargement of the liver and spleen are also common. The most suggestive tetrad is hydrocephalus, convulsions, chorio - retinitis and cerebral calcification. The cerebro-spinal fluid is frequently xanthochromic, with raised protein content and an increase in the number of cells.

The disease may also be acquired and present as a typhus-like illness, which may be fatal. It should be suspected in patients with fever, a maculo-papular rash and symptoms of encephalitis, pneumonia and sometimes myocarditis.

More recently it has been recognized that an acquired infection may closely resemble infectious mononucleosis with fever, lymphadenopathy, lymphocytosis and sometimes atypical mononuclear cells in the blood, with a negative result to the Paul-Bunnell test. Siim (1951, 1952) in Copenhagen has described eight such cases, and Cathie (1953) in London has described six. Both these workers have

[1] Received on December 24, 1956.
[2] Pathologist, Footscray and District Hospital.

isolated the organism from the saliva of some of their patients.

Finally, the most recently recognized form of acquired infection is one in which myocarditis is the single presenting manifestation (Paulley *et alii*, 1954, 1956).

As would be expected in view of the protected form of the organism in chronic infections, the acute acquired infections are the ones which are most likely to respond to treatment. A great variety of therapeutic agents have been tested in experimental infections (Eyles, 1953), and some control has been obtained by the use of sulphones; "Aureomycin" has also been used with some success, but in both instances the disease usually recurs on cessation of treatment. The most promising drug combination for experimental work has been sulphadiazine and "Daraprim", both in maximum tolerated doses, but acute human infections have been too infrequent to provide material for clinical trials of these drugs.

Diagnostic Tests

The diagnosis of toxoplasmosis depends almost always on serological tests, as it is very difficult to isolate the organism by animal or egg inoculation, either from post-mortem tissue or from the living patient. A skin test may also be used, but it has no advantage over the other tests and has the disadvantage of requiring a further visit by the patient for the reading of the test. There are two serological tests which must be used in conjunction to make a diagnosis of active toxoplasmosis. One is a complement-fixation test, which follows the usual procedure of such tests; the antigen is prepared from ten-day-old chick embryo chorioallantoic membranes which have been inoculated with mouse peritoneal exudate containing toxoplasmas and incubated for five days. The second test is called the dye neutralization test or cytoplasm-modifying test, which depends on the presence of a different antibody from the complement-fixing antibody. Toxoplasmas will take up an alkaline methylene blue stain and appear as blue ovoid or crescentic bodies; but if the dye is added to them in the presence of antibodies, they remain unstained or, in other words, their cytoplasm is modified by the antibodies.

The cytoplasm-modifying antibodies appear in the serum within two weeks of the onset of an infection, and rise rapidly to a high titre. They can pass readily across the placenta, so that a single positive result from the test on a newborn baby's serum is not indicative of congenital infection. There is no certainty as to the length of time the antibodies remain in a patient's blood, but they remain there for many years and possibly for life. The complement-fixing antibodies are of more help in assessing the activity of an infection. They appear at a slower rate than the cytoplasm-modifying antibodies, and possibly disappear from the serum more quickly, although Cathie and Cecil (1954) believe that the titre and the duration of these antibodies in the serum are merely a reflexion of the severity of the infection. The best indication of an acute infection is a rising titre in the dye test and a negative finding in the complement-fixation test, which later becomes positive.

Incidence in Australia

Toxoplasmosis has been recognized in Australia since 1946 (Robertson, 1946), mainly in the congenital form, and most of the cases have come to the attention of ophthalmologists (Hertzberg, 1952). In New South Wales two or three cases have been diagnosed each year in babies and young children, and further cases have been reported in South Australia (Swan and French, 1956), Western Australia (Edmonds, 1949) and Victoria (Jack, 1952). In New South Wales one probable case of myocarditis has been found during life in a child of six years; the diagnosis was not suspected for some weeks after the onset of the illness, when the result of the dye test was positive to a titre of 1 : 512. A month later it had fallen to 1 : 64. On both occasions the result of the complement-fixation test was positive to a titre of 1 : 8. So far there have been no cases reported of the type of illness that resembles glandular fever. The disease has also been reported in Australian dogs, cats and sheep (Wickham and Carne, 1950).

Antibody surveys in many countries have shown that subclinical toxoplasmosis is much more common than overt infection; it was therefore decided to investigate the incidence of latent and asymptomatic toxoplasmosis in a sample of the Australian population. For this purpose the dye test was used as a screening test, as the result of the complement-fixation test is never positive in the presence of a negative result from the dye test.

The sera tested were obtained from 1000 persons of various ages. Those between the ages of eighteen and sixty years were blood donors at the Red Cross Blood Transfusion Service; some of the young adults were patients attending the ante-natal clinics of King George V Hospital in Sydney and Footscray and District Hospital in Melbourne, and a few samples were obtained from National Service trainees. The children's sera were selected

from children admitted to the Royal Alexandra Hospital for Children, Sydney, for such conditions as appendicitis, fractures and other specific and well diagnosed illnesses.

Several modifications of the dye test have been described in the literature, particularly by Sabin and Feldman (1948) and by Cathie and Cecil (1954); the one used in this investigation followed that described by the latter workers. The organisms were obtained from the peritoneal exudates of mice which had been inoculated with toxoplasmas three days previously. Older exudates were not used, as four-day-old and five-day-old exudates were found to contain an increasing number of premodified organisms. The most satisfactory methylene blue preparation was found to be an aqueous solution of the dye of pH 11 which had ripened for twenty-four hours. No saline or water was used anywhere in the test, the only diluting fluid being human serum containing "accessory factor". This is an unidentified component of serum related to but not identical with complement, the presence of which is necessary to allow antibodies to modify the cytoplasm of the toxoplasmas and prevent them from taking up the dye. A preliminary step was to test the sera of the laboratory staff for the possessors of a high concentration of accessory factor; this was not easy, as only about 40% of persons have enough of the substance to allow high titres to be obtained with known positive sera, and some of these have antibodies as well, and are therefore unsuitable. Two satisfactory donors were obtained, and some 200 millilitres of serum were obtained from each, sealed in ampoules and stored at −20° C.

TABLE I

Age Group (Years)	Number	Percentage of Positive Results
0 to 9	93	8
10 to 19	187	18
20 to 29	190	25
30 to 39	240	32
40 to 49 ..	180	32
50 and over ..	110	34

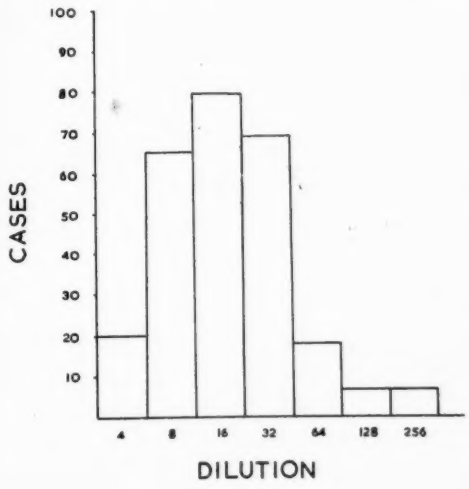


FIGURE I

The actual test consisted of making serial dilutions of a patient's serum, adding an equal volume of peritoneal exudate diluted to contain 20 to 30 organisms per high power field, and incubating the mixture for one hour. An equal volume of methylene blue solution was then added, and the tubes were examined microscopically with the high power as soon as possible. If no antibodies are present, at least 95% of the organisms in each of the tubes are stained blue and become round or ovoid instead of crescentic. When antibodies are present, the organisms appear as colourless crescents. The titre is the tube in which at least 50% of the organisms in each high power field are unstained. Table I shows the results obtained in the different age groups. There is an increase in the number of positive results obtained from childhood to young adulthood, with a further insignificant rise in late middle age to a maximum of 35%. Most of these results were in the titre range of 1 : 8 to 1 : 32; a few were positive to a titre of 1 : 4, while some had a higher titre still (Figure I).

DISCUSSION

The dye test has been widely used to determine the incidence of latent infection in many countries, and the results have all shown that, while clinical manifestations are relatively uncommon, asymptomatic infections are very frequent. Cathie and Dudgeon (1953) found that 43% of the adult population had cytoplasm-modifying antibodies, while Beverley and Beattie (1954) found them in 25% of the adults in Sheffield. Feldman (quoted by Weinman, 1952) detected 70% of positive sera in persons over fifty years of age, and like Cathie he found that the incidence in the various age groups rose progressively from childhood to old age. These high figures are not due to lack of specificity of the antibodies, as the only organism related antigenically to *Toxoplasma gondii* is the rare sarcosporidia.

The incidence of latent infection in New South Wales and Victoria is lower than in America but very similar to the figures of Beverley and Beattie in England. Until more is known about the method of spread of the disease and the reservoir of infection, the reasons for the different frequencies can only be matters for conjecture. It is possible that in some areas the wild and domestic animals are the source of infection, which may be transmitted to humans either by the ingestion of infected flesh or by the handling of infected skins. Beverley and Beattie (1954) have presented convincing evidence of the prevalence of infection among handlers of infected animals. On the other hand, the identification of toxoplasmas in saliva indicates that salivary contamination may also be a prevalent method of spread and one that is likely to affect a wider section of the community. Salivary gland infections are being investigated at the present time, but to date there are insufficient figures to indicate how important a source of infection in this country these cases may be.

The high incidence of antibodies in the population gives a positive finding from the dye test much the same significance as a positive Mantoux reaction. It emphasizes the importance of carrying out the complement-fixation test in suspected cases and at the same time observing variations in the titres at a later date.

Acknowledgements

I am greatly indebted to Dr. R. J. Walsh, Director of the New South Wales Red Cross Blood Transfusion Service, who provided me with most of the sera used in the survey. Thanks are also due to the honorary staff of the Royal Alexandra Hospital for Children, King George V Hospital and Footscray and District Hospital, who allowed me to test the sera of their patients. Dr. E. French, of the Walter and Eliza Hall Institute, very kindly carried out complement-fixation tests for me.

References

Beverley, J. K. A., and Beattie, C. P. (1952), "Human Toxoplasma Infection", *J. Hyg.*, **52**, 37.

Cathie, I. A. B., and Dudgeon, J. A. (1953), "Interpretation of Test for Toxoplasmosis", *Gt. Ormond St. J.*, **5**, 13.

Cathie, I. A. B., and Cecil, G. W. (1954), "Some Laboratory Observations on Toxoplasmosis", *Gt. Ormond St. J.*, **7**, 25.

Edmonds, A. R. (1949), "Human Toxoplasmosis with Report of a Case", *M. J. Australia*, **1**, 456.

Eyles, D. E. (1953), "The Present Status of the Chemotherapy of Toxoplasmosis", *Am. J. Trop. Med. & Hyg.*, **2**, 429.

Hertzberg, R. (1952), "Toxoplasmosis: A Review with a Report of Three Cases", *M. J. Australia*, **2**, 45.

Jack, I. B. (1952), "Toxoplasmosis: A Review of the Literature and Report of a Case", *M. J. Australia*, **1**, 391.

Paulley, J. W., Jones, R., Green, W. P. D., and Kane, E. P. (1954), "Myocardial Toxoplasmosis", *Lancet*, **2**, 624.

Paulley, J. W., Jones, R., Green, W. P. D., and Kane, E. D. (1956), "Myocardial Toxoplasmosis", *Brit. Heart J.*, **18**, 55.

Robertson, E. G. (1946), "Toxoplasmic Encephalomyelitis, with the Report of Two Cases", *M. J. Australia*, **2**, 449.

Sabin, A. B., and Feldman, H. A. (1948), "Dyes as Microchemical Indicators of a New Immunity Phenomenon Affecting a Protozoan Parasite (Toxoplasma)", *Science*, **108**, 660.

Siim, J. C. (1951), "Acquired Toxoplasmosis", *J.A.M.A.*, **147**, 1641.

Siim, J. C. (1952), "Studies on Acquired Toxoplasmosis. II. Report of a Case with Pathological Changes in a Lymph Node Removed at Biopsy", *Acta path. et microbiol. scandinav.*, **30**, 104.

Swan, C., and French, E. (1956), "A Case of Toxoplasmosis", *M. J. Australia*, **1**, 1009.

Weinman, D. (1952), "Toxoplasma and Toxoplasmosis", *Ann. Rev. Microbiol.*, **6**, 281.

Wickham, N., and Carne, H. R. (1950), "Toxoplasmosis in Domestic Animals in Australia", *Australian Vet. J.*, **26**, 1.

THE LEAD CONTENT OF BONE IN CHRONIC BRIGHT'S DISEASE[1]

D. A. HENDERSON[2] AND J. A. INGLIS[3]
From the Queensland Institute of Medical Research

SUMMARY

Some cases of chronic Bright's disease in the twenty to forty-nine years age group in Queensland can be ascribed by clinico-pathological study to known cases, such as glomerulonephritis, chronic pyelonephritis and essential hypertension. By far the larger proportion, amounting to 63% in the present series, cannot be so allotted. The bone lead content of persons in those cases of known ætiology is the same as those of persons who died without chronic Bright's disease. The bone lead content of the persons in the group of unknown ætiology is significantly higher than in these two groups. The available evidence suggests that this high lead content is due, with few exceptions, to excessive absorption during childhood. Bone lead content can be used in cases of chronic Bright's disease as a valid indication of excessive lead absorption. Its estimation may provide a clue to the ætiology of some cases of chronic Bright's disease.

IN the course of an investigation of the high incidence of chronic nephritis in Queensland (Henderson, 1955), it became necessary to attempt to discover whether any evidence of excessive lead absorption could be found in persons who had died from this cause. Fairley (1934) first suggested that estimation of the lead content of bone might be useful for this purpose. A few such estimations were made by Murray (1939), before his work was interrupted, and by Derrick (1939) with results suggestive of a high content of lead in the bones of persons dying in Queensland with contracted kidneys. The present paper reports a much larger series of estimations. It is published separately because of its probable general application in providing a clue to the cause of some contracted kidneys.

METHOD

Specimens of bone were obtained from routine autopsies performed at Brisbane Hospital and the Laboratory of Microbiology and Pathology, Department of Health, in Queensland. Specimens from Sydney, New South Wales, came from the Departments of Pathology at the Royal Prince Alfred and Sydney Hospitals. Two pieces of bone were taken at each autopsy, one from the calvarium and one from the left sixth rib in the anterior axillary line. Possible sources of contamination were identified and avoided in the mortuaries, and the bone was sent in washed bottles to the Laboratory of the Government Analyst, Brisbane, where all estimations of lead content were made by analysts experienced in the measurement of lead in biological material. A standard method was used, the bone being dry-ashed, and its lead content measured colorimetrically after dithizone extraction. It is expressed as milligrammes per 100 grammes of moist bone.

MATERIAL

Material was collected during the years 1952 to 1955. The selection of cases of chronic Bright's disease was made on autopsy reports containing a clinical or pathological diagnosis of chronic renal disease. Particular efforts were made to collect bone from subjects in the twenty to forty-nine years age group, and specimens were obtained from practically all subjects of this age coming to autopsy in Brisbane. The relatively large number of young subjects with chronic renal disease in this series is an indication of the Queensland problem. Selection was random in the older age group, and the number of cases of chronic Bright's disease in this group does not reflect the total number examined at autopsy.

Bone samples from subjects without chronic Bright's disease were collected concurrently with those from subjects with the disease in approximately equal numbers up to the middle of 1954. At that time it was considered desirable to determine the lead content of bone in a wide variety of pathological states, so the estimations were made in consecutive autopsies. In all, material was obtained from 669 autopsies in Brisbane and from 197 in Sydney. The Sydney autopsies were consecutive.

[1] Received on December 17, 1956.
[2] Former Senior Research Fellow, Queensland Institute of Medical Research.
[3] Chief Lecturer, Department of Pathology, University of Queensland.

Approximately half of the subjects with chronic Bright's disease in the twenty to forty-nine years age group were seen by one of us (D.A.H.) during life. Routine hospital records were used for the others.

BONE LEAD IN SUBJECTS WITHOUT CHRONIC BRIGHT'S DISEASE

There is a great deal of evidence (Henderson, 1954) that persons spending their childhood in Queensland during the approximate period 1880 to 1930 were exposed to a greater lead hazard in their environment than those living in other parts of the world. Consequently it was necessary to analyse results according to residence within or outside Queensland during childhood. As census returns show that some 94% of children in the birth to fourteen years age group in Queensland were born there, and as the population was relatively stable, birthplace has been used as the criterion of childhood residence.

The patients were placed into three groups: those who were born in Queensland and died in Brisbane; those who were born outside Queensland and died in Brisbane; and those who died in Sydney.

The distribution of lead content is shown for the broad age groups birth to one, one to nineteen, twenty to forty-nine and over fifty years, in Tables IA and IB. Figure I shows the mean lead content by ten-year age groups from twenty years onwards.

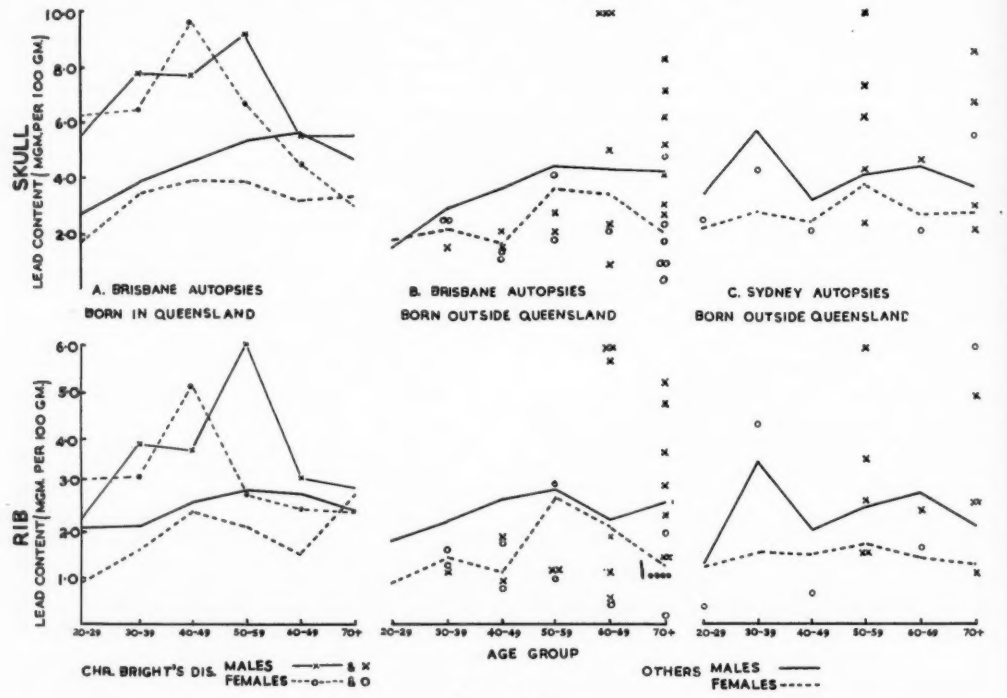


FIGURE I

Bone lead content of rib and skull in subjects aged over twenty years; *A*, who were born in Queensland and died in Queensland at Brisbane; *B*, who were born outside Queensland and died in Queensland at Brisbane; *C*, who were born outside Queensland and died in New South Wales at Sydney. Mean values in ten-year age groups are plotted throughout for subjects without chronic Bright's disease, designated "others". Values in cases of chronic Bright's disease are plotted as means in ten-year age groups for subjects in *A*, and individually for subjects in *B* and *C*

The lead content of specimens obtained from autopsies in Brisbane is similar in distribution in persons born in Queensland and those born outside this State. There is very little lead in bone in the first year of life. It increases rapidly in the first two decades and more slowly thereafter. The mean lead content of those born in Queensland is slightly higher than of those born elsewhere. This may be due to the greater childhood hazard. Female bones contain on the average less lead than male bones.

TABLE IA

Lead Content of Skull by Age, Sex and Birthplace, of Subjects Without Chronic Bright's Disease

Lead Content (Milligrammes per 100 Grammes of Moist Bone)	Brisbane Autopsies										Sydney Autopsies (Born Outside Queensland)									
	0 to 1 Year	1 to 19 Years				20 to 49 Years				50 Years and Over				0 to 1 Year	1 to 19 Years		20 to 49 Years		50 Years and Over	
	Males and Females, All Born Queensland	Males		Females		Males		Females		Males		Females		Males and Females	Males		Males		Males	
		Born Queensland	Born Elsewhere	Born Queensland	Born Elsewhere	Born Queensland	Born Elsewhere	Born Queensland	Born Elsewhere	Born Queensland	Born Elsewhere	Born Queensland	Born Elsewhere							
11·5 and over		1				1				4							1		3	1
11·4 to 10·0						1				2									4	
9·9 to 9·0						1				4							2		6	1
8·9 to 8·0						8	1			7		1					1		7	2
7·9 to 7·0					1	5	1			7	4	1	2				2		2	3
6·9 to 6·0						11	4			11	4	4					3		7	5
5·9 to 5·0		1			1	6	1	3	1	17	9	6							19	6
4·9 to 4·0		1				3	3	2		12	6	10	7				3		5	13
3·9 to 3·0		6				15	3	3	1	22	13	13	5				10		17	11
2·9 to 2·0		7		1		18	6	16	4	15	17	13	7	5			7		4	8
1·9 to 1·0		11	1	5	1	16	6	10	3	7	10	13	2		2		1			
0·9 to 0		4		4		4	3	3	5	1	2	3								
Total	24	31	1	12	2	87	25	49	15	109	75	67	30	5	2		33		68	49
Mean	0·28	2·38	1·2	1·82	1·55	3·91	3·02	3·34	1·90	5·17	4·31	3·43	3·13				3·47		4·08	2·81
Standard deviation						2·42		1·57												

TABLE IB

Lead Content in Rib by Age, Sex and Birthplace, of Subjects Without Chronic Bright's Disease

Lead Content (Milligrammes per 100 Grammes of Moist Bone)	Brisbane Autopsies										Sydney Autopsies (Born Outside Queensland)									
	0 to 1 Year	1 to 19 Years				20 to 49 Years				50 Years and Over				0 to 1 Year	1 to 19 Years		20 to 49 Years		50 Years and Over	
	Males and Females, All Born Queensland	Males		Females		Males		Females		Males		Females		Males and Females	Males		Males		Males	
		Born Queensland	Born Elsewhere	Born Queensland	Born Elsewhere	Born Queensland	Born Elsewhere	Born Queensland	Born Elsewhere	Born Queensland	Born Elsewhere	Born Queensland	Born Elsewhere							
7·0 and over		1				3	1			3	3	1					1		3	1
6·9 to 6·1						3				4							2		4	
6·0 to 5·2		1			1	3	2	1		5	7	2	3						1	1
5·1 to 4·3						14	2	2		8	9	1	4				1		8	
4·2 to 3·4		2		1		7	2	6	1	15	10	3	9				3		7	3
3·3 to 2·5		9	1	4		20	2	13	3	27	16	25	11		1		14		21	12
2·4 to 1·6		10	1	4	1	26	9	19	7	29	23	24		3	1		10		20	21
1·5 to 0·7		2		3		4	2	6	4	5	5	2					2		2	9
0·6 to 0		8																		
Total	21	31	2	12	2	87	25	49	15	109	75	67	30	3	2		33		68	49
Mean	0·28	1·71	1·1	1·37	3·05	2·44	2·36	1·84	1·21	2·80	2·59	2·04	2·19				2·09		2·69	1·60
Standard deviation						1·48		1·26												

TABLE IIA

Lead Content of Skull by Age, Sex and Birthplace, of Cases of Chronic Bright's Disease

Lead Content (Milligrammes per 100 Grammes of Moist Bone)	Brisbane Autopsies										Sydney Autopsies			
	1–19 Years		20–49 Years				50 Years and Over				20–49 Years		50 Years and Over	
			Males		Females		Males		Females					
	Males Born Queensland	Females Born Queensland	Born Queensland	Born Elsewhere	Born Queensland	Born Elsewhere	Born Queensland	Born Elsewhere	Born Queensland	Born Elsewhere	Males	Females	Males	Females
18·0 to 14·0	—	—	—	—	3	—	3	2	—	—	—	—	—	—
12·4 to 11·5 — —	—	—	2	—	2	—	—	—	—	—	—	—	—	—
11·4 to 10·5	—	—	5	—	2	—	—	1	—	—	—	—	1	—
10·4 to 9·5	—	—	3	—	2	—	4	—	—	—	—	—	—	—
9·4 to 8·5	—	—	7	—	4	—	—	—	3	—	—	—	1	—
8·4 to 7·5	—	—	4	—	2	—	1	1	—	—	—	—	—	—
7·4 to 6·5	—	—	—	—	5	—	2	1	1	—	—	—	2	—
6·4 to 5·5	—	—	4	—	3	—	4	1	3	—	—	—	1	1
5·4 to 4·5	—	1	2	—	1	—	5	2	1	1	—	—	1	—
4·4 to 3·5	—	—	2	—	3	—	3	1	3	1	—	1	1	—
3·4 to 2·5	—	—	4	—	3	—	2	3	3	1	—	1	1	2
2·4 to 1·5	—	—	3	3	1	3	1	2	4	3	—	1	2	1
1·4 to 0	1	—	—	—	—	1	—	1	—	3	—	—	—	—
Total	1	1	36	3	31	4	25	15	18	9	—	3	10	4
Mean	0·2	4·6	7·31	1·86	7·97	1·82	7·07	5·90	4·67	2·36	—	3·03	5·65	3·32
Standard deviation ..	—	—	3·10	—	3·92	—	—	—	—	—	—	—	—	—

TABLE IIB

Lead Content of Rib by Age, Sex and Birthplace, of Cases of Chronic Bright's Disease

Lead Content (Milligrammes per 100 Grammes of Moist Bone)	Brisbane Autopsies										Sydney Autopsies			
	1–19 Years		20–49 Years				50 Years and Over				20–49 Years		50 Years and Over	
			Males		Females		Males		Females					
	Males Born Queensland	Females Born Queensland	Born Queensland	Born Elsewhere	Born Queensland	Born Elsewhere	Born Queensland	Born Elsewhere	Born Queensland	Born Elsewhere	Males	Females	Males	Females
13·5 to 10·0	—	—	—	—	1	—	1	1	—	—	—	—	—	—
9·9 to 9·0	—	—	1	—	1	—	1	—	—	—	—	—	1	—
8·9 to 8·0	—	—	1	—	2	—	1	—	—	—	—	—	—	—
7·9 to 7·0	—	—	—	—	—	—	2	1	1	—	—	—	—	1
6·9 to 6·1	—	—	1	—	—	—	1	—	—	—	—	—	—	—
6·0 to 5·2	—	—	5	—	4	—	1	2	—	—	—	—	—	—
5·1 to 4·3	—	—	2	—	3	—	1	1	2	—	—	1	1	—
4·2 to 3·4	—	—	6	—	4	—	3	1	1	—	—	—	1	—
3·3 to 2·5	—	1	9	—	7	—	4	1	4	1	—	—	3	—
2·4 to 1·6	—	—	5	1	8	2	7	2	3	1	—	—	3	1
1·5 to 0·7	—	—	6	2	1	2	2	5	6	5	—	2	1	2
0·6 to 0	1	—	—	—	—	—	—	1	1	2	—	—	—	—
Total	1	1	36	3	31	4	24	15	18	9	—	3	10	4
Mean	0·2	2·9	3·48	1·2	4·14	1·4	4·21	3·46	2·53	1·23	—	2·10	3·26	2·85
Standard deviation ..	—	—	2·03	—	2·66	—	—	—	—	—	—	—	—	—

This may be due to the greater possibilities of exposure of males, for example in industrial activities, or to difference in bone composition, but the matter was not investigated.

Throughout the age groups in both sexes, the mean lead content of rib is less than that of skull in the proportion of approximately two to three. The major factor responsible appears to be difference in the proportions of inorganic material, the mean ash content of six specimens each of rib and skull being 38·2% and 51·6% respectively.

It is necessary, before ascribing any ætiological significance to a raised lead content of bone in patients with chronic Bright's disease in Queensland, to show that persons without the disease have a bone lead content which is not significantly lower than that in outside communities. This was done by obtaining specimens of bone from autopsies performed in Sydney, New South Wales. The results of analysis are contained in Tables IA and IB and Figure I. Birthplaces were obtained for the first 70 of the individuals concerned, and, as only one was born in Queensland, it was assumed that an insignificant number of members of the whole Sydney series were born in Queensland.

The mean lead content of bone from the Sydney autopsies was of the same order as that from Brisbane autopsies and, in this series, a little lower.

It is concluded that the lead content of bone of persons without chronic Bright's disease who have lived their lives in Queensland is of the same order as those who have lived outside this State.

BONE LEAD IN SUBJECTS WITH CHRONIC BRIGHT'S DISEASE

Tables IIA and IIB and Figure I show the lead content of skull and rib in persons who died of chronic Bright's disease. In the case of subjects who were born and died in Queensland, mean values have been plotted in the figure for each age group, as the numbers are fairly large, namely: males, 8, 16, 12, 10, 9, 6; females, 4, 12, 15, 4, 9, 5. The mean content of both skull and rib in males and females is approximately twice that of subjects without chronic Bright's disease up to the fifty to fifty-nine years age group. After this, it falls to the level of subjects without the disease.

As numbers are small, individual values have been plotted for persons who were born outside but died in Queensland and for those who died in Sydney. It is interesting to note that the high figures occur chiefly in males, suggesting the operation of an occupational factor.

Tables IIA and IIB show that the mean bone lead contents of the 36 males and 31 females who were born and died in Queensland aged twenty to forty-nine years were: skull, 7·31 in males and 7·97 in females; rib, 3·48 in males and 4·14 in females. From Tables IA and IB the mean values for persons in the same age group without chronic Bright's disease were: skull, 3·91 in males and 3·34 in females; rib, 1·48 in males and 1·26 in females.

As the variances increase with the means, comparisons were carried out using logarithms of the lead contents. In the log scale the variance was stable. Table III contains the mean logarithms (lead content), their differences, and the significance of those differences. The ratio differences of the lead contents are: males, skull 2·02, rib 1·45; females, skull 2·65, rib 2·47.

It is concluded that the lead content of bone in subjects aged twenty to forty-nine years who were born and died in Queensland of chronic Bright's disease is significantly higher than in subjects without this disease.

THE ÆTIOLOGICAL DIAGNOSIS OF CASES OF CHRONIC BRIGHT'S DISEASE

"Chronic Bright's disease" is a generic term comprising a number of different ætiological entities. The significance of the lead content of bone in relation to the Queensland problem can be assessed only by analysing the cases of

TABLE III

Mean Log (Lead Content) for Rib and Skull in Subjects Aged Twenty to Forty-nine Years With, and Without, Chronic Bright's Disease, who were Born and Died in Queensland

Group	Skull		Rib	
	Males	Females	Males	Females
With chronic Bright's disease ..	0·807	0·840	0·457	0·543
Without chronic Bright's disease ..	0·501	0·416	0·293	0·150
Difference	0·306±0·048	0·424±0·060	0·164±0·055	0·393±0·066
Significance	0·1%	0·1%	1·0%	0·1%

chronic renal disease in detail as to cause. The twenty to forty-nine years age group was chosen for analysis, because the greater part of the excess mortality in Queensland occurs in this group (Henderson, 1955).

Clinical and pathological diagnoses were first made separately, and final diagnosis was made by discussion. The clinician had prior knowledge of the bone lead content in some cases, but attempted to remain unbiased. The pathologist had no prior knowledge of either clinical history or bone lead content until final diagnosis was reached.

Outside Queensland, most cases of chronic renal disease can be allotted by clinical and pathological features to defined and generally accepted ætiological groups. In this series, only 12 cases could be so allotted, five to chronic glomerulonephritis, three to chronic pyelonephritis, and four to hypertension.

In the sense that there was no definite evidence that they belonged to one of the generally recognized ætiological groups, 47 cases were recorded as being of " undetermined " cause. For the purpose of the classification, lead poisoning was not regarded as one of the generally recognized causes of chronic renal disease.

One case was separated from the larger group of undetermined ætiology because of marked unilateral renal contraction, the severity of the disease being approximately equal in the two kidneys in the remainder. No histological sections were available from seven cases.

Chronic Glomerulonephritis

Five cases were identified as chronic glomerulonephritis. In one, the patient had died of cerebral hæmorrhage with no history of nephritis, and the diagnosis was made on histological grounds only. Two ran courses with initial œdema, gross albuminuria and lowered serum protein content passing into a terminal phase with hypertension and progressive renal failure, in which œdema disappeared. One case occurred in association with osteomyelitis. There was prolonged hypertension and albuminuria, but no other clinical features were present to provide a diagnosis, which was made on histological grounds. The fifth patient died of uræmia and severe hypertension nine years subsequent to an attack of acute nephritis following scarlet fever.

Histologically, all cases showed considerable destruction of glomeruli, accompanied by general parenchymal reduction and fibrosis consistent in severity with the degree of glomerular damage and extending throughout the kidney, though not necessarily uniformly. The damaged glomeruli in all these cases could still be recognized as having suffered from a glomerulonephritic process; the changes, except in one case, consisted mainly of hyaline obliteration of glomerular tufts, epithelial proliferation and crescent formation making a minor part of the picture.

Chronic Pyelonephritis

Three cases were identified as chronic pyelonephritis. Histologically, they showed characteristic patches of severe cortical atrophy and fibrosis, corresponding to areas of earlier suppuration, together with persistent traces of infection, particularly peripelvic round-cell infiltration. A firm clinical diagnosis could not be made in any of the three. In retrospect, one was of the " salt losing " type.

Hypertension

Four cases were identified as primarily of hypertensive origin. Histologically, they presented well developed lesions of arterioles and interlobular arteries, such as occur in hypertensive nephrosclerosis. They were not to be separated sharply from cases which showed similar lesions in the group regarded as being of unidentified ætiology. Macroscopically, the degree of contraction of the kidneys was much less than in the latter group. Diagnosis was based on the clinical picture, which was that of essential malignant hypertension, with recent development of severe hypertension with very high diastolic pressure, papillœdema and subsequent progressive impairment of renal function.

Cases of Undetermined Ætiology

The ætiology could not be determined in 47 cases. The clinical and histological features of this group will be described in detail in a later paper. Clinically they could be labelled only as " hypertension with renal failure ". The patients had no history of acute nephritis, of long-standing urinary infection, or of œdema prior to terminal cardiac failure. The urinary findings provided no indication of ætiology. Several had gout, which was obviously not the cause of the renal lesion.

Histologically, proliferations of capsular epithelium and adhesions of glomerular tuft to capsule were observed in some cases. Usually these changes were recent and minor. For this reason they were considered to be incidental and not related to the cause of the long-standing contraction and fibrosis or to the long-standing clinical disease. In a few cases with severely

contracted kidneys, these lesions were of sufficient severity and duration to suggest a histological diagnosis of chronic glomerulonephritis. However, indisputable histological evidence was lacking, the diagnosis was not supported by the clinical history, and after a survey of the whole series it was clear that these were properly placed with cases of unidentified origin and not with those of glomerulonephritic origin. The significance of this glomerulitis will be discussed in the later paper. Some other cases histologically resembled hypertensive nephrosclerosis, but the kidneys showed unusually severe contraction, while clinically there was evidence of long-standing renal disease without severe hypertension.

The general histological picture in the group is one of marked disappearance of renal tissue without indication of cause, changes such as those mentioned above being superimposed on the remaining parenchyma.

A few cases had clinical evidence of urinary infection during the terminal illness, and histological changes of recent infection. They were not regarded as chronic pyelonephritis, as there was neither clinical nor histological evidence of long-standing infection.

Clinically, the seven cases without histological sections came into the "hypertension with renal failure" category.

Bone Lead Content

Of the 13 subjects with chronic Bright's disease of known cause, five were females. The mean lead contents for the whole group were: skull 3·52 milligrammes per 100 grammes and rib 1·70 milligrammes per 100 grammes. The means for subjects without chronic Bright's disease shown in Tables IA and IB were: skull 3·45, and rib 2·22. The number is not large, but it appears that bone lead content in persons with chronic Bright's disease of known cause is the same as in persons without the disease.

Four of the seven cases of chronic Bright's disease in patients born outside Queensland could be given an ætiological diagnosis. They comprised three cases of chronic pyelonephritis and one primarily due to hypertension. The mean lead contents were: skull 2·0, rib 1·25.

There were 25 females and 22 males in the group in which no ætiological diagnosis was made. The essential details about these are recorded in Table IV. Mean values were: skull, 8·45 in males and 8·66 in females; rib, 4·09 in males and 4·53 in females.

It is concluded that the lead content of bone in subjects who were born and died in Queensland of chronic Bright's disease of unknown cause is significantly higher than in those who died without the disease. The lead content of persons with chronic Bright's disease of known cause is of the same order as those without the disease.

ORIGIN OF THE HIGH BONE LEAD

There are three possible causes of the high bone lead in these cases: (i) As all subjects had chronic renal disease, retention of normally absorbed lead by failing kidneys may have been responsible. (ii) There may have been some disturbance of metabolism, whereby a larger proportion of normal lead intake was absorbed. (iii) There may have been excessive absorption of lead due to excessive exposure.

There are a number of reasons why the first possibility should be discarded:

1. Including those born outside Queensland, there are 21 subjects, some with very contracted kidneys, whose bone lead is normal. In the older age group there were more such cases. This is not conclusive, as the degree of accumulation of lead in the skeleton would depend on the duration of renal failure, which is unknown. However, it is very suggestive, as some of the subjects with normal bone lead content had very contracted kidneys, probably indicating prolonged renal impairment.

2. A more fundamental reason is that there is, in fact, no significant difference between the lead excreted by the normal and by the failing kidney. Two groups of data demonstrate this. The first was obtained from Murray's monograph (1939). He published the results of a large number of urinary lead estimations in different pathological conditions. After excluding cases of plumbism, there are 35 patients regarded as having no renal disease, and 25 diagnosed as having chronic nephritis with recorded evidence of renal failure in the form of a greatly raised blood urea content. The range of urinary lead content in the absence of renal disease was 0·01 to 0·08 milligramme per litre, with a mean of 0·030 milligramme per litre, and in the presence of renal failure 0·01 to 0·08 milligramme per litre, with a mean of 0·028 milligramme per litre. The second group of data was obtained in the present investigation. Urinary lead content was estimated in 36 males. Nineteen of these had normal renal function, as judged by normal blood pressure, absence of albuminuria and ability to concentrate urine to at least 1020 in ward tests; and the remainder had renal failure, due to various causes,

TABLE IV

Bone Lead Content of Subjects of Chronic Bright's Disease Aged Twenty to Forty-nine Years

Serial No.	Age	Sex	Kidney Weights (Grammes)		Lead Content (Milligrammes per 100 Grammes)		Ætiology	Comments
			Right	Left	Rib	Skull		
			Subjects who were born and died in Queensland					
592	20	M.	98	112	1·8	2·4	Chronic glomerulonephritis	
89	45	M.	148	168	0·8	2·8	Chronic glomerulonephritis	
79	26	M.	112	84	0·9	1·8	Chronic glomerulonephritis	
122	38	F.	127	142	0·8	3·4	Chronic glomerulonephritis	
146	27	M.	196 Cystic	196 Cystic	1·3	3·1	Chronic glomerulonephritis	
142	37	F.	Small	Small	2·3	3·7	Chronic pyelonephritis	
351	35	F.	78	266	2·6	2·7	Chronic pyelonephritis	Unilateral pyelonephritis
65	47	F.	84	84	3·2	7·8	Chronic pyelonephritis	High bone lead
71	46	M.	196	196	1·2	4·3	Essential malignant hypertension	
231	42	M.	Reduced	Reduced	2·5	4·6	Essential malignant hypertension	
679	33	F.	112	140	2·0	3·7	Essential malignant hypertension	
109	38	M.	148	160	1·6	3·1	Essential malignant hypertension	
99	39	M.	Large	Small	1·1	2·4	Unilateral renal disease	Hypoplastic kidney with malignant hypertension
134	41	F.	42	42	9·1	18·0	Undetermined	Lead poisoning, age 11 years
131	46	F.	56	56	13·5	17·0	Undetermined	Lead poisoning in childhood
225	43	F.	56	50	8·4	15·0	Undetermined	Gout for many years
107	39	F.	112	112	4·4	12·0	Undetermined	
179	45	F.	—	—	5·4	11·6	Undetermined	
96	36	F.	20	28	3·6	10·8	Undetermined	
158	40	F.	77	77	8·9	10·8	Undetermined	Gout for fourteen years
30	26	F.	112	84	5·0	10·0	Undetermined	Indefinite lead poisoning, age seven years
5	35	F.	56	60	5·6	10·0	Undetermined	Lead poisoning, age five years
35	41	F.	84	84	3·8	8·8	Undetermined	Gout for five years
101	49	F.	84	84	4·2	8·8	Undetermined	
69	49	F.	28	28	3·0	8·6	Undetermined	
40	34	F.	71	99	2·1	8·2	Undetermined	Lead poisoning in childhood
26	33	F.	56	56	2·5	7·1	Undetermined	Lead poisoning, age three years
86	39	F.	28	56	5·4	7·0	Undetermined	
226	43	F.	102	84	3·4	7·3	Undetermined	
296	48	F.	70	56	3·3	7·0	Undetermined	
628	43	F.	84	84	2·4	6·7	Undetermined	
128	47	F.	56	70	2·3	6·0	Undetermined	
9	28	F.	140	140	2·7	5·9	Undetermined	
63	33	F.	84	84	5·4	5·6	Undetermined	
78	25	F.	56	28	2·6	5·2	Undetermined	
88	25	F.	56	56	2·3	3·8	Undetermined	
70	45	F.	84	84	2·3	3·3	Undetermined	
680	36	F.	Reduced	Reduced	1·7	2·1	Undetermined	
76	30	M.	60	60	4·4	12·2	Undetermined	Industrial lead poisoning
164	49	M.	Reduced	Reduced	5·5	11·5	Undetermined	
18	36	M.	84	98	6·9	11·2	Undetermined	
34	46	M.	204	190	9·1	11·2	Undetermined	
67	40	M.	56	28	3·6	10·8	Undetermined	Gout, two years
20	31	M.	84	84	3·4	10·4	Undetermined	Gout, seven years
19	38	M.	Half-normal	Half-normal	3·4	9·7	Undetermined	
56	40	M.	63	59	2·5	9·7	Undetermined	Lead poisoning in childhood
569	28	M.	84	84	2·2	9·0	Undetermined	
22	31	M.	84	112	5·2	9·0	Undetermined	Gout, two years
68	39	M.	84	84	8·3	8·8	Undetermined	Lead poisoning in childhood
80	32	M.	56	84	4·0	8·5	Undetermined	Lead poisoning in childhood
74	43	M.	112	98	3·0	8·6	Undetermined	Gout, nine years
132	35	M.	Small	Small	2·8	8·3	Undetermined	
627	32	M.	95	110	1·0	8·0	Undetermined	
678	40	M.	Very small	Very small	5·8	7·9	Undetermined	Lead poisoning, aged two years
84	23	M.	56	84	2·5	6·4	Undetermined	
64	32	M.	84	56	5·8	6·4	Undetermined	
111	28	M.	84	70	3·3	5·8	Undetermined	Gout, fourteen years
114	40	M.	84	70	2·5	5·4	Undetermined	
110	29	M.	152	112	2·2	4·0	Undetermined	
24	37	M.	76	84	1·7	3·1	Undetermined	Lead poisoning, aged four years
518	42	F.	42	56	4·9	8·7	Undetermined	Clinically "renal failure with hypertension". No histological sections
87	35	M.	56	84	5·4	8·8	Undetermined	Clinically "renal failure with hypertension". No histological sections
90	46	M.	112	112	3·0	5·7	Undetermined	Clinically "renal failure with hypertension". No histological sections
112	47	M.	84	98	4·3	11·2	Undetermined	Clinically "renal failure with hypertension". No histological sections
301	31	M.	112	112	3·3	7·5	Undetermined	Clinically "renal failure with hypertension". No histological sections
234	38	M.	196	196	3·6	8·5	Undetermined	Clinically "renal failure with hypertension". No histological sections
232	28	M.	154	154	4·5	11·0	Undetermined	Clinically "renal failure with hypertension". No histological sections

TABLE IV.—*Continued*

Bone Lead Content of Subjects of Chronic Bright's Disease Aged Twenty to Forty-nine Years.—Continued

Serial No.	Age	Sex	Kidney Weights (Grammes)		Lead Content		Ætiology	Comments
			Right	Left	Rib	Skull		
Subjects who were born outside and died in Queensland								
13	36	F.	28	56	1·7	2·3	Chronic pyelonephritis	
17	36	F.	Reduced	Reduced	1·5	2·3	Chronic pyelonephritis	
133	36	M.	56	56	1·0	1·6	Chronic pyelonephritis	
106	43	M.	196	196	0·8	1·7	Hypertension	
105	43	F.	112	84	0·9	1·1	Undetermined	
68	39	M.	54	54	1·6	2·4	Undetermined	
82	42	F.	56	56	1·6	1·8	Undetermined	

evidenced by the presence of albuminuria, a fixed urinary specific gravity of 1010, and a raised blood urea content in the absence of extrarenal causes of uræmia. The mean age of those with renal failure was 43·4 years and of those without it 37·1 years. With the exception of one person who was an outpatient, all members of both groups were inpatients on normal hospital diet for at least a week before the estimations were made. The range in lead content of urine in the presence of renal failure was 0·01 to 0·07 milligramme per litre, with a mean of 0·022 milligramme per litre, and in the absence of renal failure 0·01 to 0·02 milligramme per litre, with a mean of 0·016 milligramme per litre. Thus it is evident that the concentrations of lead in the urine of persons with normal and with impaired renal function living under usual conditions of lead exposure are of the same order of magnitude.

3. In addition to the above reasons, the kidney is not the only path of excretion of lead from the body. Alternative routes exist through bowel and skin (Shiels, 1954).

In view of these considerations, it is concluded that the high lead content of bone in these cases of chronic Bright's disease is not due to the retention of normally ingested lead by failing kidney function.

The second possibility could have been tested by detailed balance studies. However, it seemed so unlikely on general grounds that it was not followed up.

With the other possibilities eliminated, only that of excessive absorption due to excessive exposure remains, and there is evidence to support it.

Of the subjects with high bone lead content, one male gave a history of industrial lead poisoning. It was impossible to check all the occupations of the remaining males for possible lead exposure, but it can be stated that, in general, there has not been much industrial plumbism in Queensland. Four males and a sibling of another had a history of lead poisoning in childhood.

There has been no industrial exposure of females in Queensland, and no other hazard to adult females has been known to exist in this State. Six of the females with chronic Bright's disease and a high bone lead content had a history of lead poisoning in childhood.

There was for many years a serious and widespread lead hazard to children in Queensland (Henderson, 1954). A number of investigators (Travers *et alii*, 1956; Bridge, 1953; Williams *et alii*, 1933) have shown that where a lead hazard exists, investigations of siblings and associates of children with plumbism will reveal many additional instances of grossly excessive lead absorption, with few, if any, acute clinical manifestations. The above facts make it most probable that the excessive lead in the bones of nearly all these young individuals with chronic Bright's disease was acquired during their childhood.

THE SIGNIFICANCE OF THE PRESENT INVESTIGATION

The results reported here do provide a link between chronic nephritis in Queensland and excessive lead absorption. This link is an important part of the circumstantial evidence incriminating plumbism as the cause of the high incidence of "chronic nephritis" in this State.

In addition to its local significance, however, we feel that the estimation of lead content of bone in chronic Bright's disease may well have a wider application. Plumbism, both industrial and non-industrial, clinical and subclinical, occurs in most civilized communities. Despite some doubt, particularly in America, there is a great deal of evidence that lead produces contracted kidneys, and a future paper dealing with the Queensland problem will add more. It is submitted that some cases of chronic

Bright's disease occurring outside Queensland may also be due to plumbism, and that the routine estimation of bone lead content at autopsy in cases of chronic renal disease without obvious cause may elucidate their ætiology. The basis for this submission is to be found in Figure I. In the group who were born outside Queensland, thereby avoiding the special hazard in childhood, a number of subjects of chronic Bright's disease had a high bone lead content. It so happens that all are males in the older age groups, which suggests an industrial exposure. Some subjects with chronic renal disease among those in the Sydney autopsies also have a high bone lead content. On the other hand, Table IV shows that occasional subjects with renal disease of known cause have a high bone lead content due to coincidental excessive exposure. As would be expected, the lead content is of ætiological significance only when considered in conjunction with clinical and histological data.

ACKNOWLEDGEMENTS

This investigation has been made possible only by the willing cooperation of many people. It is a pleasure to thank the Government Analyst, Mr. S. B. Watkins, and his staff, for performing the lead estimations, Dr. J. I. Tonge and Dr. M. J. J. O'Reilly of the Laboratory of Microbiology and Pathology, Department of Health and Home Affairs, Dr. A. W. Pound and his staff at Brisbane Hospital, Dr. V. J. McGovern of Royal Prince Alfred Hospital, Sydney, and Dr. A. W. Palmer of Sydney Hospital, for arranging the collection of autopsy material, and Mr. P. B. McGovern for statistical advice.

REFERENCES

BRIDGE, A. (1953), "Lead Intoxication in Three Families", *M. J. Australia*, **2**, 62.

DERRICK, E. H. (1939), in "Annual Report on the Health and Medical Services of Queensland for 1938–39", 60.

FAIRLEY, K. D. (1934), "A Review of the Evidence Relating to Lead as an Ætiological Agent in Chronic Nephritis in Queensland", *M. J. Australia*, **1**, 600.

HENDERSON, D. A. (1954), "A Follow-up of Cases of Plumbism in Children", *Australasian Ann. Med.*, **3**, 219.

HENDERSON, D. A. (1955), "Chronic Nephritis in Queensland", *Australasian Ann. Med.*, **4**, 163.

MURRAY, R. E. (1939), "Plumbism and Chronic Nephritis in Young People in Queensland", Commonwealth of Australia, Department of Health Service Publication (School of Public Health and Tropical Medicine), No. 2.

SHIELS, D. O. (1954), "The Elimination of Lead in Sweat", *Australasian Ann. Med.*, **3**, 225.

TRAVERS, E., RENDLE-SHORT, J., and HARVEY, C. C. (1956), "The Rotherham Lead-Poisoning Outbreak", *Lancet*, **2**, 113.

WILLIAMS, H., SCHULZE, W. H., ROTHCHILD, H. B., BROWN, A. S., and SMITH, F. R. (1933), "Lead Poisoning from Burning Battery Casings", *J.A.M.A.*, **100**, 1485.

ENDOCARDIAL FIBROELASTOSIS[1]

JOHN BEVERIDGE[2]

From the Royal Alexandra Hospital for Children and Institute of Child Health, Sydney

SUMMARY

Attention is directed to endocardial fibroelastosis as a not uncommon cause of fatal heart disease in infancy, and one which is exciting an increasing amount of interest. Details are submitted of six patients who died from acute and chronic forms of the disease. In the acute form the symptomatology is alarming. Upon the vague and non-specific background of irritability, refusal of food and restlessness, there develops a shock-like condition in conjunction with neck and limb stiffness, cyanosis and respiratory distress. Death is usual in this form. In the chronic form the clinical picture is one of congestive cardiac failure with secondary pulmonary infection. In this form cardiac murmurs are present and the electrocardiogram is grossly abnormal. The possibility of diagnosis in life and the difficulty of differentiating fibroelastosis from other forms of heart disease causing cardiac failure in infancy are discussed. The suggestion is made that, with improved diagnosis, a greater number of infants will be found to suffer from this condition and yet not die. The outlines of medical treatment and of the current palliative surgical approach are presented.

THE term "endocardial fibroelastosis" (or "E.F.E.") is chosen in this present report because it describes the gross appearance of the heart at autopsy, without defining the ætiology; it is the term in most general use, being synonymous with "endomyocardial fibrosis" (Ball, Williams and Davies, 1954), "endomyocardial sclerosis" (Watt and Lynch, 1954) and "endocardial sclerosis" (Blumberg and Lyon, 1952). The term refers to a white, opaque, fibroelastic thickening of the endocardium, which frequently involves the cardiac valves.

Fibroelastosis is not rare, almost 200 case reports having been published, but no series has yet been reported in the Australasian literature available to the writer. Blumberg and Lyon, who refer to it as "one of the commonest types of fatal heart disease in infancy", reported a series of 25 cases, and Adams and Katz (1952) another series of 21 cases. Although in many instances suspected during life, the diagnosis has usually been made at autopsy; Adams and Katz, however, after a long experience, feel confident that a clinical diagnosis can be made, and in four of their reported cases the patients are living.

Dennis, Hansen and Corpening (1953) have made a wide review of the literature and, as a result, describe three clinical types. The first of these is called fulminating, and 25% of 149 cases reported in the literature were classified in this group; the affected infants, who were all less than six weeks of age, underwent an abrupt transition from apparent normality to severe dyspnœa and cyanosis, and death followed in minutes or hours. The second type was described as acute, and 51% of the patients were regarded as belonging to this type. They were aged between six weeks and six months, and were apparently in normal health until the unexpected development of dyspnœa with cyanosis; in many instances the onset simulated an episode of choking due to the aspiration of food or vomitus. Repeated paroxysms of dyspnœa with cyanosis followed, and between these paroxysms the features of the illness were irritability, anorexia, cough and tachycardia; X-ray examination of the chest showed a globular type of cardiomegaly. None of these patients survived longer than four weeks. In the third group, called chronic, the patients were usually older infants and small children, and the onset of symptoms was relatively slow, beginning with refusal of food and irritability, followed by a "hacking" cough and stunting of growth, and at a late stage, dyspnœa and cyanosis. There was no clubbing of fingers; tachycardia was constant, but fever was absent unless an intercurrent infection developed. The electrocardiogram was "always" abnormal, showing *T*-wave depression in leads I, II, V4, V5 and V6, but never inversion of *T* waves.

[1] Received on January 3, 1956.

[2] Medical Officer, Institute of Child Health, Sydney.

Another group has been called by Horley (1955) a "fœtal" type; the patients have aortic atresia and relative or absolute hypoplasia of the left ventricle, with resultant right ventricular hypertrophy. They develop cyanotic attacks within thirty-six hours of birth, and die in the first week or two of life.

Although endocardial fibroelastosis is reported not infrequently in pædiatric journals of the United Kingdom, the United States of America, Germany and Switzerland, this condition is not confined to children, or to the white races. Watt and Lynch refer to the condition occurring in adults, and Ball *et alii* report 20 cases, the youngest patient being two years of age and the oldest eighty-four years, all of these patients being African natives.

MATERIAL

Six patients who died in the Royal Alexandra Hospital for Children during the years from 1951 to 1954 were shown at autopsy to have endocardial fibroelastosis.

Reports of Cases

CASE I (Case No. 3650).—M.K., a female, aged two years, was admitted to hospital on December 6, 1951, and died on December 12, 1951. Her birth weight was eight pounds 13 ounces. The mother's pregnancy had been normal. The patient had two normal siblings, older than the patient.

It was noted that three months prior to admission to hospital the patient's respirations had been abnormal and grunting in character. About six weeks later, oliguria became obvious, urine being passed but twice daily. Shortly after this she began to have cyanotic episodes, and between episodes was unusually pale; for the last four days prior to admission to hospital all these symptoms had been worse. In addition, she developed œdema of her feet, a slight dry cough and orthopnœa.

When admitted to hospital, she had pallid cyanosis and pitting œdema of the legs and back, and the respirations were more rapid than usual (40 per minute), as was the pulse rate (130 per minute). Both radial and femoral pulses were palpable, and the systolic blood pressure was 110 millimetres of mercury, but no assessment could be made of the diastolic level. The apex beat was palpable in the fourth left intercostal space, just external to the midclavicular line. A harsh systolic murmur was audible, the maximum intensity being at the apex. No abnormality was found on examination of the lungs, but the abdomen was distended from ascites (circumference 21 inches), The urine was acid in reaction and normal to routine chemical testing.

The red blood cells numbered 4,930,000 per cubic millimetre. The hæmoglobin value was 11·7 grammes *per centum*. The white cells numbered 10,400 per cubic millimetre, being made up of neutrophile cells 6448, lymphocytes 3328, monocytes 312, eosinophile cells 208, and basophile cells 104 per cubic millimetre. The serum protein content was 6·3 grammes *per centum* (albumin 4·9, globulins 1·4). The blood urea content was 44 milligrammes per 100 millilitres. Microscopic examination of urine revealed five to 12 leucocytes per high-power field. The sedimentation rate was four millimetres in one hour. X-ray examination of the chest (Figure I) showed the heart shadow to be grossly enlarged and globular in shape; there was possible partial collapse of the lower lobe of the right lung, but the vascularization of the lung fields appeared to be within normal limits.

Autopsy (No. 6607A) was performed on December 13, 1951. There was generalized œdema of subcutaneous tissues, with considerable ascites and small hydrothoraces; the lungs were heavy from congestion and œdema, and liver and spleen were slightly enlarged as a result of venous congestion. There was a moderate

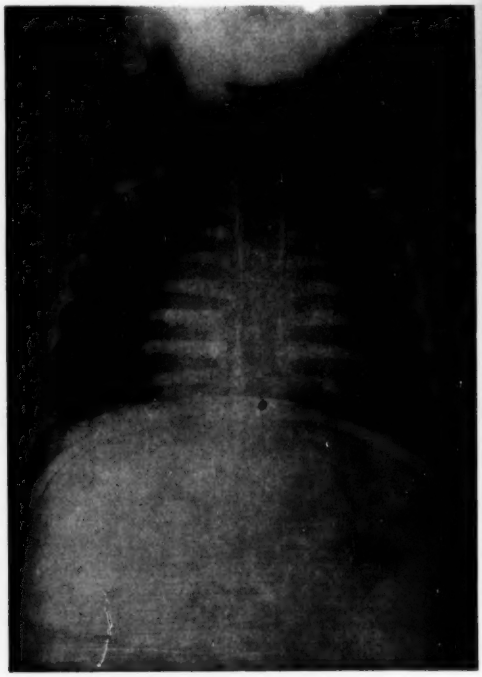


FIGURE I

excess of pericardial fluid. The great vessels were normal, and the *ductus arteriosus* was closed. Both auricular chambers were appreciably increased in size, and the right ventricle was slightly dilated but not hypertrophied. The endocardium of both auricles was thickened, and all valves except the pulmonary valve were abnormal. The mitral valve was the most grossly affected, being stenosed to such a degree that only the tip of the little finger was admitted; the cusps were short, of extremely dense consistency and pearly white, and a lesser degree of fibrotic contracture had spread down the *chordæ tendinæ* to involve the papillary muscles. The tricuspid valve was very similar, but the communication between the auricle and ventricle was larger on this side. The three semilunar cusps of the aortic valve were thickened in a uniform fashion. Microscopic examination of sections of the heart showed fibrous thickening of the mitral valve and considerable fibrous and elastic hypertrophy

of the endocardium of the left auricle and ventricle, with stout bands of fibrous tissue spreading outwards into the inner half of the ventricular wall and the wall of the right auricle.

CASE II (Case No. 4486).—C.M.R., a female child, aged four months, was admitted to hospital on January 18, 1952, and died on the same day. Her

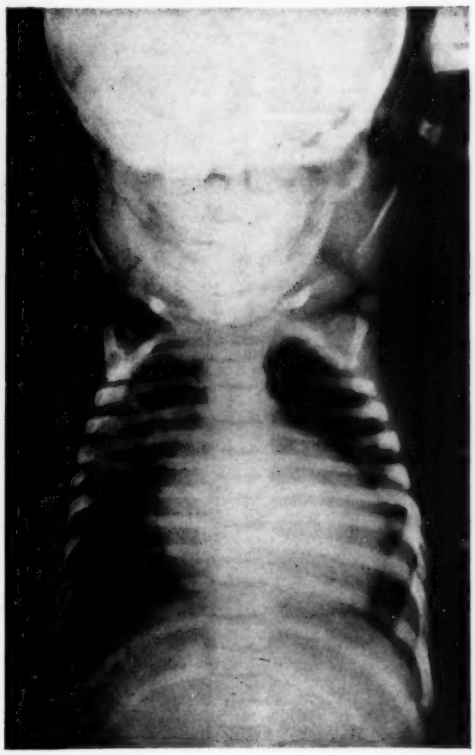


FIGURE II

birth weight was six pounds. The mother's pregnancy had been normal; it was the first pregnancy after ten years of marriage. The child had no siblings. She had been perfectly well until January 13, 1952, when, during her breast feeding, she stiffened, arched her back and became pale, and then grey and clammy, and her breathing was laboured; the whole episode lasted thirty minutes. Similar episodes occurred on January 14 and January 16; there were three episodes on January 17 and two on January 18 prior to admission to hospital. During all of this time she had been accepting feedings poorly. When admitted the infant was poorly nourished (her weight was eight pounds seven ounces) and had pallid cyanosis; the respiration rate was 80 per minute and the heart rate 160 per minute. Peripheral arterial pulses could not be felt, and blood pressure could not be recorded. There was no peripheral œdema. The heart was enlarged to clinical examination with an apical systolic thrill and a harsh systolic murmur of great intensity at the apex. The lungs were normal to clinical examination, and the liver was not palpably enlarged. Despite oxygen therapy, the infant's condition deteriorated rapidly, and she died a few hours after admission; the temperature rose to 106° F. just prior to death.

The red blood cells numbered 3,470,000 per cubic millimetre. The hæmoglobin value was 10·6 grammes *per centum*. The white cells numbered 16,100 per cubic millimetre, being made up of neutrophile cells 10,465, lymphocytes 5313 and monocytes 322 per cubic millimetre. X-ray examination of the chest (Figures II and III) showed an enlarged globular heart with consolidation in the upper lobe of the right lung.

Autopsy (No. 6657A) was performed on January 19, 1952. The lungs were very congested, and microscopic examination of sections showed congestion and œdema. The liver showed no evidence of chronic congestion.

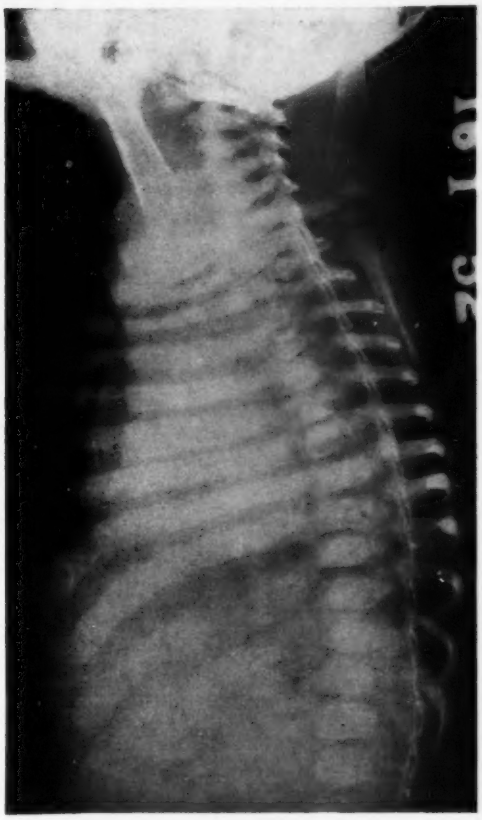


FIGURE III

The great vessels were normal, but the *ductus arteriosus* was patent. The heart was moderately enlarged owing to dilatation of the auricles and right ventricular hypertrophy; the mitral valve orifice was narrowed by fibrous thickening of the valve ring and cusps. The *chordæ tendinæ* were short and fibrous, and there was endocardial thickening in the left auricle and, to a lesser extent, in the left ventricle. Histological

examination of these parts confirmed the presence of excess fibrous and elastic tissue, and the appearances suggested that the process was still active.

CASE III (Case No. 810).—J.P., a male child, was born on June 2, 1952. He was first admitted to hospital on August 5, 1952, at the age of two months,

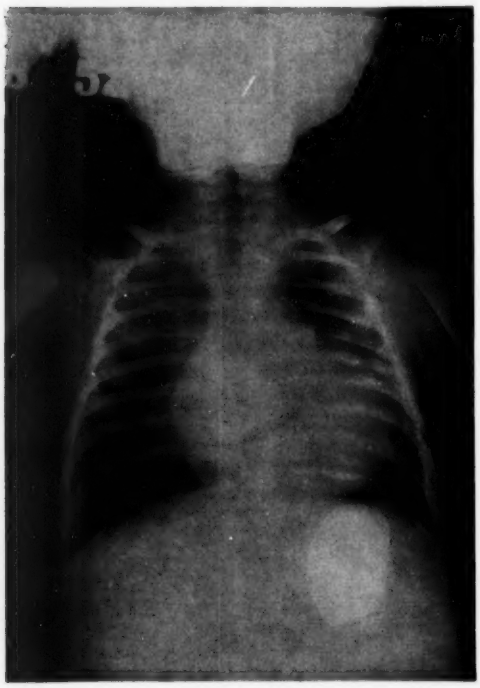


FIGURE IV

and discharged on September 26. He was again admitted on October 12 at the age of four and a half months and discharged on October 29. His third admission was on November 10 at the age of five months, and he was discharged on November 24. He was finally admitted on December 3 at the age of six months, and died on December 3, 1952.

This child came to the hospital from a hostel controlled by the Child Welfare Department; no details of his history were available except he had had respiratory distress and appeared unwell. His weight at the time of his first admission was eight pounds three ounces; no abnormality was found on clinical examination at first, but just prior to his discharge from hospital a soft systolic murmur was heard, one centimetre internal to the apex beat, and, at the same time, it was noted that the femoral pulses were absent. He took feedings well, gained weight, and on discharge from hospital showed no symptoms. Investigations performed at this admission showed a total red blood cell count of 3,210,000 per cubic millimetre. The hæmoglobin value was 10·8 grammes *per centum*. The white cells numbered 8300 per cubic millimetre, being made up of neutrophile cells 3569, lymphocytes 3818, monocytes 830 and basophile cells 83 per cubic millimetre. X-ray examination of the chest (Figures IV and V) showed a globular enlargement of the heart with normal lung fields. The electrocardiogram demonstrated (Figure VI) inversion of *T* waves in leads I, II, aVL and aVF, and præcordial leads V_2 to V6.

After his first discharge from hospital the child remained well until October 11, 1952, when he developed tachypnœa and dyspnœa and was re-admitted to hospital. His weight was then only nine pounds fourteen ounces. His temperature was 101° F. Considerable respiratory distress was present with a respiration rate of 60 per minute. Rales were heard at both lung bases. The apex beat was one centimetre external to the mid-clavicular line, the heart rate was 220 per minute, and a systolic murmur was again heard. The liver edge was felt two centimetres below the subcostal margin in the midclavicular

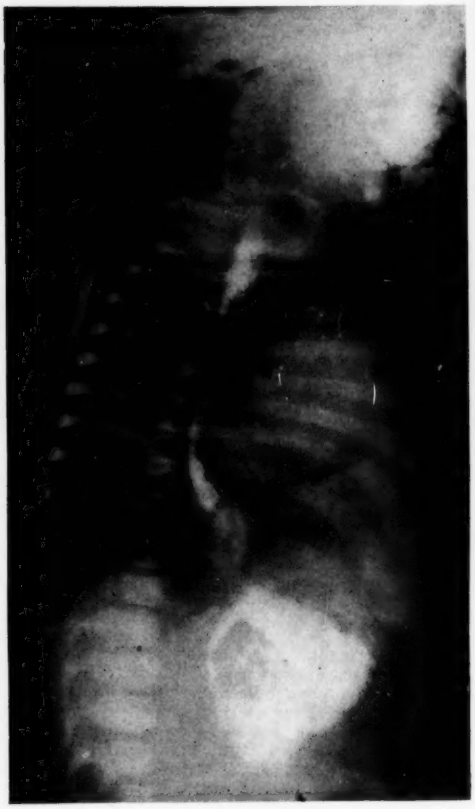


FIGURE V

line. He was placed in an oxygen tent, was given digitalis and antibiotics, and improved rapidly during the next twenty-four hours. All treatment was stopped on October 20, and thereafter he remained asymptomatic until he was discharged back to the hostel. Investigations performed at this admission included an X-ray examination of the chest (Figure VII) which indicated further cardiac enlargement.

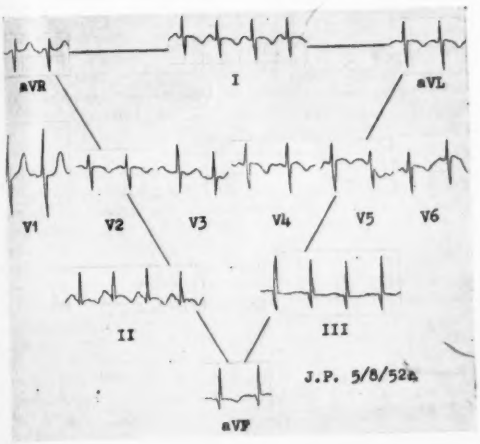


FIGURE VI

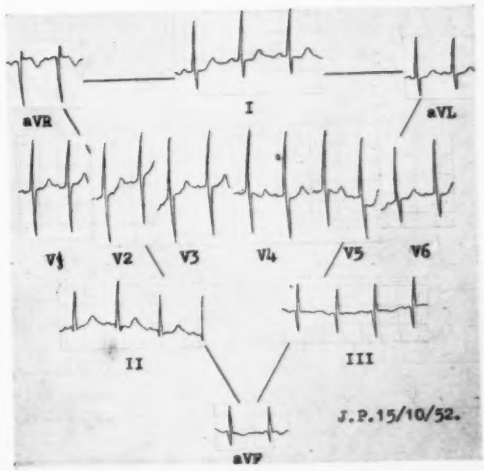


FIGURE VIII

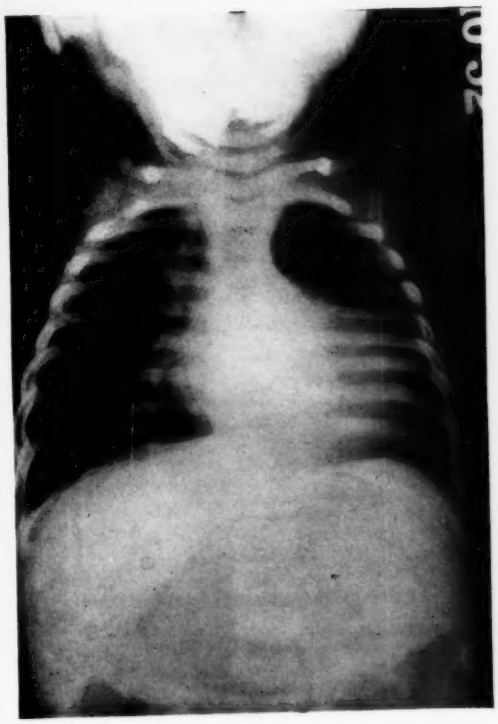


FIGURE VII

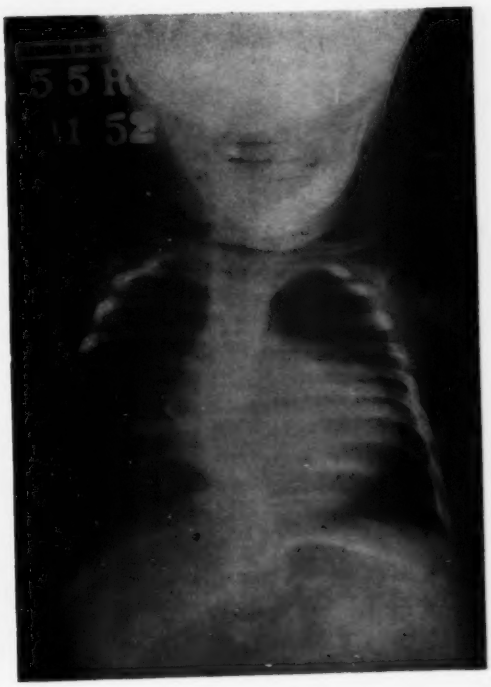


FIGURE IX

In the electrocardiogram (Figure VIII) the *T* waves were normal (by October 15 full digitalization had been achieved).

After a further period of observation as an outpatient, the baby was admitted to hospital for a third time on November 10, having become ill a few hours

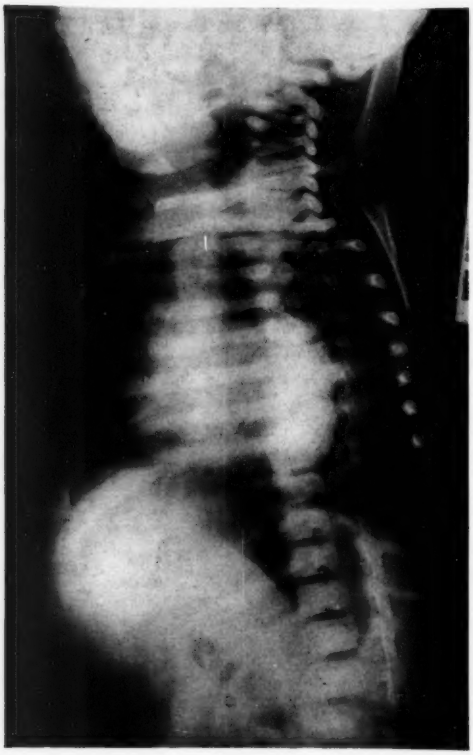


FIGURE X

before with restlessness and rapid, shallow and difficult respirations with periods of cyanosis. When he was admitted, his respiration rate was 70 per minute and his temperature was normal. Some rhonchi were heard over both sides of his chest. The radial pulses were easily felt, but not the femoral pulses; the apex beat was in the midclavicular line, and a systolic murmur was audible, being loudest at the apex. The liver edge was barely palpable. He was given penicillin for six days and again improved rapidly, being free of symptoms when discharged. During this admission X-ray examination of the chest (Figures IX and X) showed that the heart size was unchanged, but there was an increase in the lung markings at the right lung base. The electrocardiogram (Figure XI) demonstrated inversion of *T* waves in leads I, II, III, aVF and V6.

At his final admission to hospital it was learnt that the child had developed, quite suddenly, severe respiratory distress, tachypnœa and cyanosis a few hours previously. He had pallid cyanosis, a respiratory rate of 60 per minute, crepitations audible over both sides of the chest, tachycardia (200 per minute), clinical enlargement of the heart, an apical systolic murmur, and a liver palpable three centimetres below the subcostal margin in the midclavicular line. He was given oxygen and penicillin, and died two hours after admission to hospital.

Autopsy (No. 7202A) was performed on December 4, 1952. The lower lobe of the left lung was partially collapsed as the result of pressure exerted by the heart, which was considerably enlarged, mainly because of left ventricular hypertrophy. The right ventricle and both auricles were dilated. The endocardium lining the left side of the interventricular septum was considerably thickened, and this thickening involved, to a lesser degree, the remainder of the left ventricle, the mitral valve and the left auricle. Only one coronary orifice was present, and the vessel concerned divided into right and left coronary arteries soon after its origin from the aorta; the aorta itself was considerable narrowed between the left subclavian artery and the *ductus arteriosus*, which was closed. Histological examination confirmed the considerable thickening of the endocardium of the left auricle and ventricle, and showed that the lining of the right ventricle was involved to a slight degree.

CASE IV (Case No. 4121).—B.F., a male child, was born on July 7, 1952. He was admitted to hospital on January 27, 1953, at the age of six months, and died on April 24, 1953. His birth weight was seven pounds eleven ounces. His mother's pregnancy had been normal. He had no siblings. Three weeks before admission to hospital he had been irritable for a period of twenty-four hours, but thereafter had been well

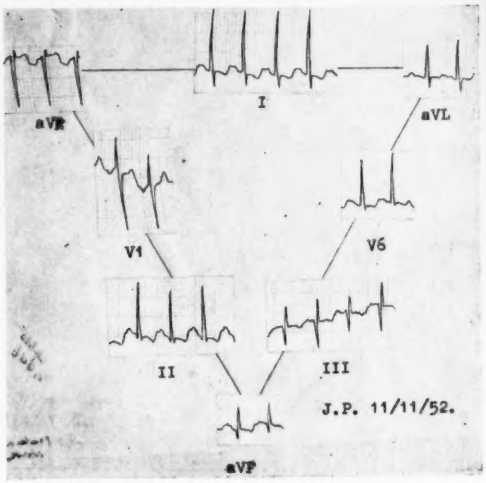


FIGURE XI

until January 25, 1953. He then became irritable and restless, sleeping fitfully, refusing both breast and solid feedings, and developing a slight cough. Examination confirmed the marked irritability. The temperature was 104° F. There was neck and spine stiffness. Some decrease in intensity of the breath sounds was noted over the lower lobe of the left lung. The cardio-vascular system was thought to be normal. Cerebro-spinal fluid obtained by lumbar puncture was

opalescent, but when lumbar puncture was repeated on the following day, cerebro-spinal fluid was normal. On January 29 the apex beat had moved to the left as far as the midclavicular line, a soft systolic murmur could be heard at the apex, the liver was slightly enlarged, there was moderate respiratory distress with rales audible over both sides of the chest, and signs suggesting consolidation of the lower lobe of the left lung. Concurrently with the administration of penicillin and streptomycin, the temperature fell to normal.

Thereafter his clinical condition deteriorated until February 8 when, with increasing signs of congestive cardiac failure, dyspnœa and occasional cyanosis, administration of digitalis was commenced. This resulted in some improvement. When this improvement was not sustained, administration of " Mersalyl " was begun on February 13, but no improvement resulted. Thereafter the baby's state became progressively worse, with increasing dyspnœa and cough and more frequent cyanosis; in addition to the presence of rales on both sides of the chest it became

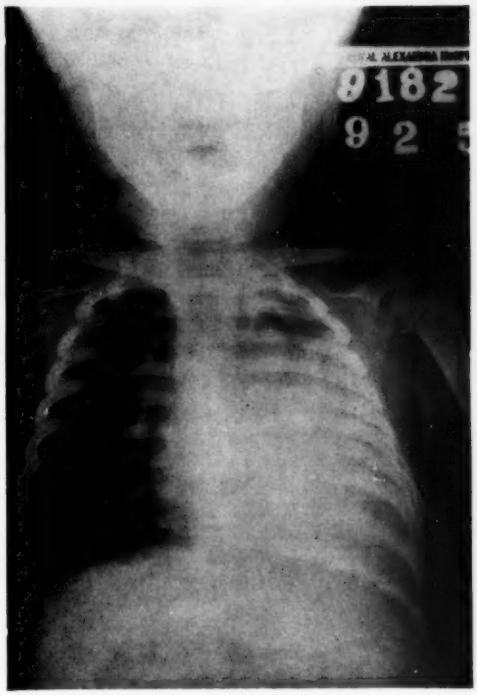


FIGURE XII

clear that the lower lobe on the left side was completely collapsed. On April 24 the temperature was again raised, and cyanosis became constant despite the administration of oxygen. The baby died on April 25.

Examination of the cerebro-spinal fluid on January 27, 1953, showed 80 cells per cubic millimetre (polymorphonuclear cells 70%, lymphocytes 30%), and 40 milligrammes of protein and 750 milligrammes of chloride per 100 millilitres; glucose was present. On January 28, 1953, the cerebro-spinal fluid contained three cells per cubic millimetre (polymorphonuclear cells 30%, lymphocytes 70%), and 10 milligrammes of protein and 720 milligrammes of chloride per 100 millilitres; glucose was present. The result of the Mantoux test (1 : 1000 old tuberculin) was negative. The hæmoglobin value was 10 grammes *per centum*.

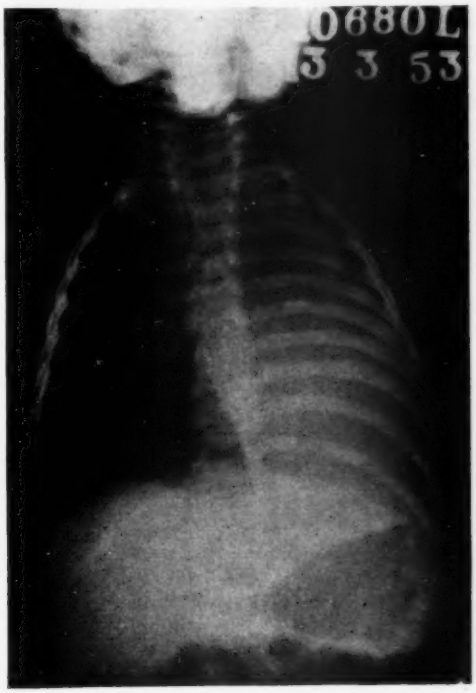


FIGURE XIII

The white cells numbered 27,000 per cubic millimetre, being made up of neutrophile cells 16,008, lymphocytes 9660 and monocytes 1932 per cubic millimetre. X-ray examination of the chest (Figures XII and XIII) showed globular enlargement of the heart with progressive collapse of the lower lobe of the left lung. An electrocardiogram (Figure XIV) showed *T* waves inverted in leads I, II and V6, and of low voltage in lead III, and unipolar limb leads.

Autopsy (No. 7467A) was performed on April 27, 1953. The entire left lung was collapsed, as a result of compression of the left main bronchus, and the right lung was congested. The liver was enlarged from venous congestion. The heart was grossly enlarged and occupied most of the left pleural sac; the great vessels were normal, and the *ductus arteriosus* was closed. The enlargement was due, for the most part, to hypertrophy and dilatation of the left ventricle, and the endocardium of this ventricle was much thickened and opaque; the mitral valve was slightly sclerosed, as was the endocardium of the left auricle. The aortic valve and the right side of the heart were normal. Microscopic examination of sections merely confirmed the fibroelastic thickening of the endocardium.

CASE V (Case No. 10,139).—K.D., a female child, was born on March 21, 1952, admitted to hospital on January 1, 1954, at the age of twenty-one months, and died on the same day. Her birth weight was five pounds eight ounces. Her mother's pregnancy had been normal, except for delivery one month prematurely. During the week before admission to hospital she had suffered from a slight wheeze. At 6 p.m. on December 29, 1953, she had, without warning,

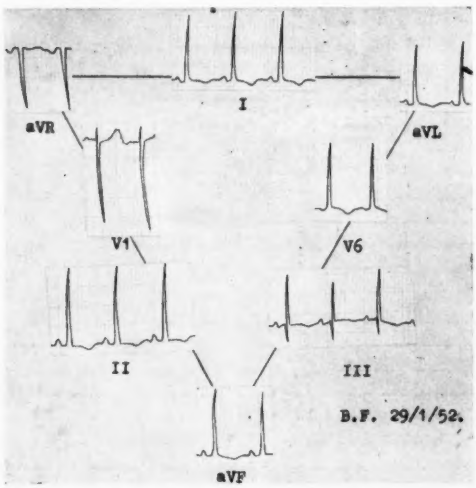


FIGURE XIV

a " convulsion " becoming pale, stiff and unconscious, but with no twitching. This episode lasted about two minutes, but immediately afterwards she appeared to be normal. An hour before admission to hospital she again lost consciousness, and developed opisthotonus and twitching of her arms. She was still unconscious when admitted, with pallid cyanosis, tachypnœa (respiration rate 40 per minute) and tachycardia (160 per minute). The heart and lungs appeared to be normal. A lumbar puncture was performed immediately, and she died a few minutes later. The cerebro-spinal fluid was normal; no other investigations were undertaken.

Autopsy (No. 7930c) was performed on January 4, 1954. The right pleural cavity was half filled with a clear straw-coloured fluid, and the left contained a lesser amount; the lungs were congested. The liver was moderately enlarged as a result of congestion. The great vessels appeared normal, and the *ductus arteriosus* was closed. The heart was considerably enlarged from dilatation and hypertrophy of both auricles and ventricles, more marked on the right side. The endocardium was of a glistening white appearance throughout most of the left auricle and ventricle, and to a lesser extent in the right auricle and ventricle. The mitral and tricuspid valves were normal, but the cusps of the aortic valve were of a fleshy thickness. Microscopic examination confirmed the endocardial thickening and muscle hypertrophy, and showed the coronary vessels to be normal.

CASE VI (Case No. 11,188).—P.M., a male child, was born on June 22, 1952, was admitted to hospital on February 26, 1954, at the age of nineteen months, and died on the same day. His birth weight was eight pounds thirteen ounces. His mother's pregnancy had been normal. He had one normal sibling, aged six years. At the time of admission to hospital he was desperately ill. He had been sick for six weeks. During the initial period of the illness symptoms had been vague—refusal of food, irritability (particularly at night), pallor and a disinclination to stand or walk. For the week before his admission to hospital all these symptoms had been noticeably worse; he had also developed a short dry cough, and occasionally he had vomited. Respirations were said to be " grunting " in character, and cyanosis of the lips was noted on February 26 for the first time. When first seen the boy was moribund. He was stuporose, and moderately cyanosed. No peripheral pulses could be felt, and no estimate could be made of the blood pressure. The respirations were rapid (70 per minute), and coarse rales and rhonchi were audible over the whole of the chest, so that no appreciation of the heart sounds could be made. The liver could be felt easily, its edge being four centimetres below the subcostal margin in the midclavicular line; it felt hard and smooth. Despite treatment with antibiotics and oxygen, he died fifty minutes after admission to hospital. His hæmoglobin value was 12·0 grammes *per centum*. A

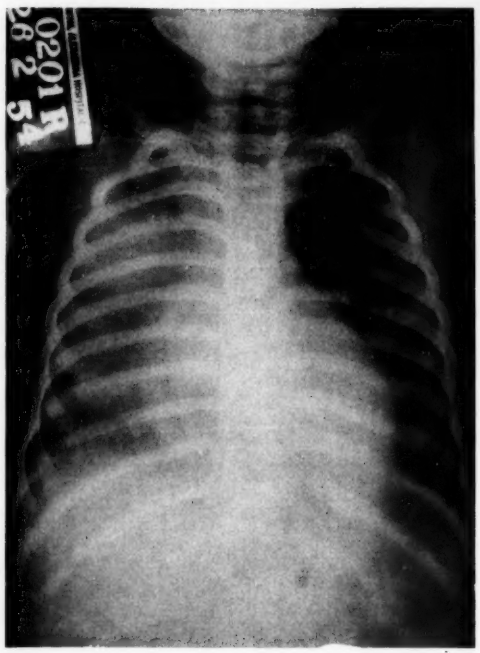


FIGURE XV

white cell count showed a total of 56,000 per cubic millimetre, made up of neutrophile cells 34,720, lymphocytes 15,680 and monocytes 5600 per cubic millimetre. X-ray examination of the chest (Figure XV) showed that the heart was somewhat enlarged, with partial opacity veiling the right lung.

Autopsy (No. 8041c) was performed on February 27, 1954. Patchy areas of consolidation were present in both lungs, and the left lower lobe was completely

consolidated; microscopic examination showed extensive bronchopneumonic consolidation. The liver and spleen were much increased in size from venous congestion. The great vessels were normal, and the *ductus arteriosus* was closed; there was an excessive amount of straw-coloured fluid in the pericardial sac, which otherwise appeared normal. The heart itself was greatly enlarged as a result of dilatation and some hypertrophy of the left ventricle, but the heart muscle and coronary arteries appeared normal. The endocardium of the right auricle and ventricle was normal, but in the left auricle and ventricle it was greatly thickened and whitish in appearance; the cusps of the mitral and aortic valves were thickened. Microscopic examination of sections from the interventricular septum and the wall of the left ventricle showed pronounced thickening of the endocardium of the left ventricle (Figure XVI); the muscle fibres were hypertrophied, with groups of chronic inflammatory cells between them, but without increase in interfascicular fibrous tissue. These appearances justified a diagnosis of interstitial myocarditis with fibroelastosis of the endocardium (Figure XVII).

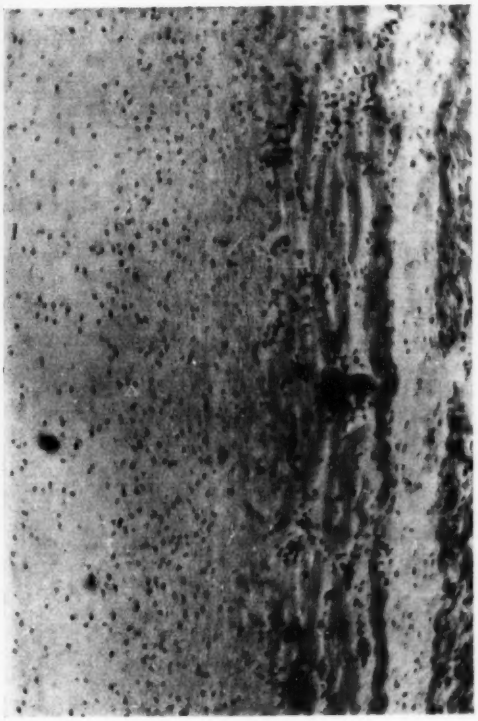


FIGURE XVI

DISCUSSION

Classification

None of these patients had the sudden, catastrophic type of illness described by Dennis *et alii* as "fulminating", for, although three patients survived for less than twenty-four hours after reaching hospital (C.M.R., K.D. and P.M.), it was clear in each case that some symptoms had been present for at least a few days, but the significance of such symptoms had not been recognized. Two of these patients (C.M.R. and K.D.) survived for less than a

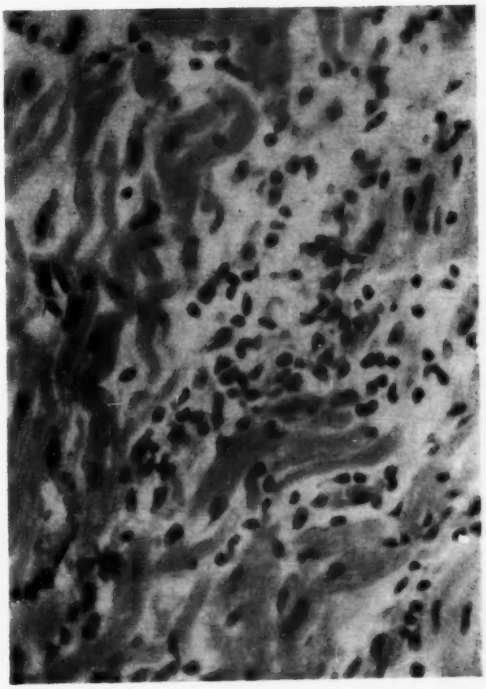


FIGURE XVII

week after the onset of symptoms, and the illness might reasonably be called acute. The third patient in this group had symptoms for six weeks prior to admission to hospital, which suggests that this illness should be classified as "chronic", but the rapid deterioration in the few days before reaching hospital and the fact that death occurred within an hour of his admission render such a term unsuitable. It is of some interest to note that one patient, whose illness does not fall easily into either the acute or the chronic groups, had, in addition to fibroelastosis, evidence of interstitial myocarditis; this combination of disorders has not been previously recorded in the literature as far as can be determined, and may be the explanation of this minor difficulty of clinical classification.

The other three patients (M.K., J.P. and B.F.) had long illnesses, at least three months

in duration, but it is of interest that one of them (M.K.), in spite of obvious and severe symptoms, was not presented for admission until six days before death. Another patient (J.P.) improved clinically so much on three occasions that it was deemed unnecessary to keep him continuously in hospital.

Clinical Features

Kempton and Glynn (1954) have pointed out that symptoms can be divided into two groups: (i) symptoms due to cardiac failure, (ii) symptoms due to secondary infection.

The symptomatology of the two patients classed as having an acute illness and of the third patient in the "borderline" nuance was alarming in the extreme. Upon the vague and non-specific background of irritability, refusal of food and restlessness, there develops, suddenly and frighteningly, a shock-like condition, characterized by disturbance of consciousness, stiffness of the back and limbs, cyanosis and respiratory distress, any of which may be very marked. These dramatic episodes, terrifying to the medical observer and even more dreadful for the parents of the child, last for a few minutes or as long as an hour, and are repeated at intervals which may vary from an hour to a day or so. These three patients, when admitted to hospital, were already near death; they were unconscious, and in a shock-like state. Only in the patient whose illness was of longer duration (P.M.) was there evidence of congestive cardiac failure; a second patient (C.M.R.) obviously had a cardiac abnormality, though its exact nature was not capable of clinical definition; the third patient (K.D.) showed no evidence of cardio-respiratory disease on clinical examination.

The other three patients whose illnesses have been classified as chronic presented the picture of congestive cardiac failure, with secondary pulmonary infection in two of them. Again there is the vague background of irritability and feeding difficulty, but thereafter the course is slower and not nearly so dramatic as in the acute group. They developed cough, difficulty with breathing even to the point of orthopnœa, and at times fever, which seemed to be caused by secondary lung infection or by absorption collapse due to compression of the left main bronchus. All of these children showed cyanosis at some time during the course of their illness, and were all of them cyanosed when death was near. Only one of them (M.K.) exhibited those episodes of pallid cyanosis and shock which occurred in each of the patients in the acute group. A similar comment on cyanosis has already been made by Dennis *et alii*, but other writers have not made such an observation; Blumberg and Lyon, for example, regard cyanosis as a terminal event only, in both acute and chronic forms of the disease.

These three patients with the chronic type of illness all had significant systolic murmurs, and in one of them (M.K.) the murmur was very loud and harsh. Another (J.P.) was suspected of having a coarctation of the aorta, because of the inequality of the radial and femoral pulses; and, in addition, because of the grossly abnormal electrocardiogram, some congenital anomaly of the coronary circulation was postulated. Freer and Matheson (1953), by contrast, did not find murmurs in any of their patients.

In all three patients there was evidence of right-sided heart failure, as well as pulmonary congestion and œdema; although, as is at times seen in infancy, the sole evidence for right-sided heart failure in two of them was enlargement of the liver without elevation of the jugular venous pressure or peripheral œdema. One child (M.K.) was exceptional in having marked peripheral œdema and ascites. These findings correspond closely with those of Kempton and Glynn, but Dennis *et alii*, in their review of published cases, found that hepatomegaly was present in only 15% of cases.

Radiological Features

Fortunately, it was possible to obtain some radiographic studies in all children except one, who died too soon after admission to hospital for X-ray films to be undertaken. In two patients it was possible to obtain X-ray pictures of the chest at intervals over periods of three months. As a rule, this examination helps to establish that there is a cardiac abnormality, in that it shows the heart to be enlarged and globular in shape, but there is nothing in the radiological appearances which is diagnostic of fibroelastosis. Since it is often difficult, if not impossible, to demonstrate enlargement of the heart in infants by clinical means, these studies are of great value. All five patients showed definite radiological evidence of cardiac enlargement, and, in addition, the films were of value in demonstrating associated or secondary pulmonary consolidation, collapse and congestion.

Electrocardiographic Findings

Most writers have little to say concerning the electrocardiographic findings; Lyon and Kaplan (1954) state that the electrocardiograph

is usually abnormal but not pathognomonic of fibroelastosis. These authors describe tall *R* waves and inversion of *T* waves in the left ventricular surface leads, and occasional alterations of the *S-T* segments. It is hardly surprising that a great number of observations is not available, for in the fulminating and in the acute types, time does not permit of electrocardiographic investigation. Horley does not record any electrocardiographic findings for the three patients with the fœtal type of disease which he has described; Blumberg and Lyon are of the opinion that there is no constant or typical pattern; Ball *et alii* describe conduction defects. The best study is that of Dennis *et alii*, who are of the opinion that the electrocardiogram is always abnormal, at least in the chronic type of illness, with depression but never inversion of the *T* waves in leads I and II, and in the left ventricular surface leads.

In the six cases recorded here, electrocardiography was performed on only two patients, each of whom was under observation for three months (J.P. and B.F.). The prominent abnormality is the marked inversion of *T* waves in leads I and II, and in the left ventricular surface leads. It is necessary to call attention to two facts in relation to the findings in one of these patients (J.P.). Firstly, this patient had an anomalous origin of the coronary arteries, in that both arose from a common trunk, which in turn arose from the aorta, together with an infantile type of coarctation of the aorta. Secondly, the *T* waves in this electrocardiogram became normal when he had been digitalized (Figure VI). Gross abnormalities occur in the electrocardiograph when the left coronary artery arises from the pulmonary artery, but this is essentially a different anomaly of coronary circulation from that present in this patient. The other child (B.F.), however, had no abnormality of aortic valves or aorta, and the origin of the coronary arteries was normal.

Autopsy Findings

Enlargement of the heart with evidence of pulmonary congestion and œdema are constant findings, except in the fœtal type, where there is aortic atresia with hypoplasia of the left ventricle. In approximately half the reported cases, evidence of right ventricular failure is also present. All writers are agreed that the thickening of the endocardium is more often found in the left side of the heart than on the right; Dennis *et alii* found that 98% of patients reported in the literature had involvement of the lining of the left ventricle. In approximately half the patients, valvular deformities have been found, again principally in the left side of the heart. These deformities include complete atresia of the aortic valves in the fœtal form described by Horley, as well as stenotic lesions of any or all of the four valves, and in the mitral and tricuspid valves the disease process not uncommonly spreads to affect the *chordæ tendineæ* and papillary muscles.

Another, but not frequent, finding of interest, and of possible ætiological significance, is the association of endocardial fibroelastosis with certain congenital defects. In addition to aortic atresia, it is recognized that fibroelastosis is found with coarctation of the aorta and with anomalous origin of the left coronary artery from the pulmonary artery with a greater frequency than can be explained by chance.

Analysis of the findings in the six patients here described reveals that in all of them there was thickening of the endocardium of the left auricle and left ventricle, in four the mitral valve was stenosed and in three the aortic valve was stenosed, whereas in only two was the right side of the heart the site of endocardial thickening. In no case was the pulmonary valve diseased. One patient had coarctation of the aorta together with an anomalous origin of the coronary arteries from a common trunk, and in another patient (P.M.) interstitial myocarditis was found in association with fibroelastosis.

Diagnosis

It is unusual to make a definite diagnosis of endocardial fibroelastosis in life, but Adams and Katz, in their series of 21 patients, include four patients who were still living and for whom this diagnosis had been made with reasonable certainty. Paul and Robbins (1955), in outlining the surgical management of patients with this condition, record the histories of four living patients, one of whom died subsequent to operation, with autopsy confirmation.

Diagnosis in the fœtal type can be suspected when an infant has cyanotic episodes beginning within thirty-six hours of birth, and this suspicion is strengthened if all the peripheral pulses are absent. In the fulminating group, the patient is not seen until after death; the diagnosis can only be retrospective, and can, at best, be suspected in a very young infant who dies unexpectedly in a paroxysm akin to choking.

In the acute type of disease, it should be more easily possible to make a diagnosis, provided there is an awareness of this condition; shock-like episodes, with disturbance of consciousness and pallid cyanosis, together with the

demonstration by radiography of a globular form of cardiomegaly, are very suspicious of endocardial fibroelastosis.

A diagnosis of the more chronic form of this illness will be entertained when symptoms of left-sided heart failure and evidence of right-sided heart failure are associated with a globular enlargement of the heart, and with electrocardiographic abnormality, particularly *T*-wave changes in leads I and II and in the left ventricular surface leads. Other conditions causing cardiac enlargement with congestive heart failure are capable of limited differentiation, and, in particular, one should remember coarctation of the aorta, anomalous origin of the left coronary artery from the pulmonary artery, the cardiac form of glycogen storage disease, gargoylism and idiopathic myocarditis.

Coarctation of the aorta in its various forms, with and without patency of the *ductus arteriosus*, is too well known to warrant description here. Anomalous origin of the left coronary artery from the pulmonary trunk, which is considerably less common than fibroelastosis, and to which references have already been made, causes failure of both sides of the heart, marked cardiac enlargement, and marked electrocardiographic changes, characterized by a deep, wide *Q* wave, elevation of *S-T* segments and inversion of *T* waves over the left ventricular surface leads. It is clear that differentiation of the two conditions is difficult, and this difficulty is increased by the combination, at times, of the two conditions in the same patient. Fortunately, at the present time the separation of one from the other is not of practical importance, since not only is the medical management much the same, but the palliative surgical treatment which will be described for fibroelastosis can be applied also to patients with the anomaly of the left coronary artery.

The cardiac form of glycogen storage disease, though much less common than fibroelastosis, is similar in its clinical manifestations. Glycogen storage disease gives rise to symptoms at birth or within the first six to eight months of life, and presents in the form of cardiac failure with or without heart murmurs, globular enlargement of the heart and changes in the *S-T* segments and *T* waves of the electrocardiogram. Since the cardiac form of glycogen storage disease is fundamentally different from the hepatic form, blood sugar estimations, response of the blood sugar to adrenaline and other metabolic tests are of no help in the diagnosis of the cardiac form. Biopsy of skeletal muscle may, on some occasions, be of help if an excess of glycogen can be demonstrated in the muscle.

In gargoylism, symptoms of heart failure and of coronary insufficiency do not usually develop until the second decade. Should enlargement of the heart with electrocardiographic changes be found early in life, the other evidence of gargoylism is so strong that no confusion with fibroelastosis should arise. Idiopathic myocarditis in infancy is more common than endocardial fibroelastosis, and an excellent report of 14 cases in Melbourne has been published by Williams, O'Reilly and Williams (1953). Infants with myocarditis present with symptoms of pulmonary congestion and œdema, and develop signs of right-sided heart failure; there are usually no murmurs to be heard; if present, they sometimes fluctuate in intensity. Enlargement of the heart, though uncommonly apparent on clinical examination, can be demonstrated by X-ray examination, with congestion of the lung fields. Dennis *et alii* have attempted to distinguish idiopathic myocarditis from fibroelastosis by means of the electrocardiographic tracings, stating that inversion of *T* waves in the left ventricular surface leads does not occur in fibroelastosis, but other writers do not agree, and experience at the Royal Alexandra Hospital for Children suggests that the electrocardiographic changes in fibroelastosis are more definite than in idiopathic myocarditis. Many infants with idiopathic myocarditis, if treated adequately during the severe, early stage of the illness, tend to improve thereafter, and gradually return towards normality (although some permanent damage may be left); whereas in patients with the chronic form of fibroelastosis, the trend is towards progressive deterioration, punctuated at times by febrile complications. The course of the illness, then, is possibly the best guide to the differentiation of the two conditions. However, Adams and Katz, who also have experienced difficulty in distinguishing one from the other, postulate that some patients with fibroelastosis do survive the initial cardiac failure and proceed to live a relatively normal life, though the heart may remain permanently enlarged and structurally altered.

Prognosis and Treatment

Many a medical condition has been recognized, at first, as a pathological entity at autopsy, with the conclusion drawn that such a disease is uniformly fatal; but after analysis of the clinical features associated with such a pathological finding, it has become possible to entertain the diagnosis before death, and finally

to make a confident diagnosis in patients who then survive the onslaught of the disease. This historical pattern may well be followed in the evolution of our understanding of fibroelastosis. Although nearly all the first 200 cases (including the six recorded here) have been reported after death, in some patients the diagnosis has been suspected before death, autopsy confirming the diagnosis, and seven infants were still living when their details were reported.

Since, in the chronic form of fibroelastosis, the prognosis is probably not always fatal, these patients deserve careful medical management of the congestive cardiac failure and its complications. This is now largely standardized, but, although Nadas, Radolph and Reibbold (1953) prefer digitoxin, experience at the Royal Alexandra Hospital for Children in the management of cardiac failure in infancy due to any condition has been that digoxin is effective and the dosage and effect can be controlled easily. The dose of digoxin used to achieve digitalization within twenty-four hours has been 0·070 milligramme per kilogram of body weight, given in divided doses over the first twenty-four hours; thereafter, to maintain the effect, a useful guide to dosage has been 0·007 to 0·020 milligramme of digoxin per kilogram of body weight per twenty-four hours, the actual dosage being determined after due consideration of the effects of the drug on the particular patient. A low sodium diet can be achieved for even the smallest infants by replacing the usual feeding (no matter whether breast or artificial feeding) with a dried milk of low sodium content of which a preparation is now available commercially. Administration of oxygen, mercurial diuretics, mechanical aids (venesection, aspiration of hydrothoraces) and the use of antibiotics for the infective pulmonary complications have all been useful.

"*Poudrage*" has been the subject of a report by Paul and Robbins; their technique is to dust four to five grammes of sterile talc into the pericardial sac, which has been laid open at thoracotomy, with the idea that the fine particles of magnesium silicate will cause an adhesive granulomatous inflammation with new vessels to anastomose with those of the myocardium. Of their four patients so treated they claim that three showed clinical improvement, although heart size was not reduced.

Ætiology and Pathogenesis

The findings in the cases presented here shed no new light on the problem of ætiology, which has been the subject of much speculation. Horley postulates that, at least for the fœtal type, anoxia of the endocardium and myocardium is a possible cause, pointing out that no case of aortic atresia has been found in which there is fibroelastosis accompanying a ventricular septal defect, and conversely, that no case of aortic atresia with an intact septum was without endocardial fibroelastosis. Kempton and Glynn support the idea that the cause is either anoxia or circulatory stasis, and draw attention to the similarity of the microscopic appearance of the thickened tissue in fibroelastosis to that of the endocardial fibrosis overlying an area of cardiac infarction in an adult. Suggestions of a developmental defect of embryonic life and of intrauterine endocarditis find little favour in the literature, most writers stressing the rarity of the condition in the first two months of life. Watt and Lynch have drawn attention to the possibility of a "collagenosis" as the explanation, but this is denied vigorously by Adams and Katz. Weinberg and Himmelfarb (1943) reported fibroelastosis in siblings.

Symptoms appear to be caused by pulmonary congestion and œdema, by right-sided heart failure or by secondary infection. When the cardiac valves are structurally altered by the thickening process, it is relatively easy to see that this mechanical disorder could result in heart failure; but when the thickening is limited to the endocardium lining the heart chambers without involving the heart valves, such an explanation is inadequate, and two alternatives have been propounded. The first of these is that the thickened endocardium, particularly that lining the left ventricle, is a mechanical embarrassment to the action of the heart, splinting the myocardium and limiting its action in a manner similar to that of constrictive pericarditis. The second suggestion, which has more general acceptance, and which is supported by the electrocardiographic findings, is that the interference with the heart's action is due to myocardial ischæmia, and that this is due to obstruction of the Thebesian veins by the thickened endocardium. If this be so, then the surgical approach, as a palliative measure, and in combination with adequate medical treatment, finds justification.

ACKNOWLEDGEMENTS

It is a pleasure to record my gratitude to Dr. R. D. K. Reye, Director of the Institute of Pathology at the Royal Alexandra Hospital for Children, who stimulated my interest in this condition and gave helpful advice, and who performed the autopsies on these patients;

the illustrations were prepared for me in the department of photography, which is also under his control. My thanks are due to the honorary physicians of this hospital, who have allowed me to publish details of their patients, and to Professor Lorimer Dods and Dr. D. S. Stuckey, who have been most helpful in their criticism of this paper.

REFERENCES

ADAMS, F. H., and KATZ, B. (1952), "Endocardial Fibroelastosis", *J. Pediat.*, **41**, 141.

BALL, J. D., WILLIAMS, A. W., and DAVIES, J. N. P. (1954), "Endocardial Fibrosis", *Lancet*, **1**, 1049.

BLUMBERG, R. W., and LYON, R. A. (1952), "Endocardial Sclerosis", *Am. J. Dis. Child.*, **84**, 291.

DENNIS, J. L., HANSEN, H. E., and CORPENING, T. N. (1953), "Endocardial Fibroelastosis", *Pediatrics*, **12**, 130.

FREER, J. L., and MATHESON, W. J. (1953), "Left Auricular Enlargement in Endocardial Fibroelastosis", *Arch. Dis. Child.*, **28**, 284.

HORLEY, J. F. (1955), "Fœtal Fibroelastosis", *Brit. M. J.*, **1**, 765.

KEMPTON, J. J., and GLYNN, L. E. (1955), "Heart Failure in Infancy with Abnormalities of the Valves and Endocardium", *Quart. J. Med.*, **24**, 109.

LYON, R. A., and KAPLAN, S. (1954), "Endocardial Sclerosis", in "Textbook of Pædiatrics", by Waldo E. Nelson, 6th edition, W. B. Saunders Company.

NADAS, A. S., RADOLPH, A. M., and REIBBOLD, J. D. L. (1953), "The Use of Digitalis in Infants and Children", *New England J. Med.*, **248**, 98.

PAUL, R. N., and ROBBINS, S. G. (1955), "Surgical Treatment Proposed for Either Endocardial Fibroelastosis or Anomalous Left Coronary Artery", *Pediatrics*, **16**, 147.

WATT, J., and LYNCH, J. B. (1956), "Polyarteritis Nodosa: Its Relation to Endomyocardial Sclerosis", *Lancet*, **1**, 658.

WEINBERG, T., and HIMMELFARB, A. J. (1943), "Endocardial Fibroelastosis", *Bull. Johns Hopkins Hosp.*, **72**, 299.

WILLIAMS, H., O'REILLY, R. N., and WILLIAMS, A. (1953), "Fourteen Cases of Idiopathic Myocarditis in Infants and Children", *Arch. Dis. Child.*, **28**, 271.

AUSTRALASIAN MEDICAL PUBLISHING CO. LTD.
SEAMER AND ARUNDEL STS., GLEBE, SYDNEY